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Main Authors:	Valerio Mazzoni (FEM) and Vincenzo Verrastro (IAMB)

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1 Executive Summary

This document addresses the optimized use of alternatives to pesticides proposed for the gradual phase-out of mineral oil and reports the description of the combination of the different alternative means of disease/pest control (*C. ternatea*, orange oil, vibrational mating disruption), on greenhouse crops (tomato/pepper and cucurbits) to reduce the populations of the greenhouse whitefly (GW), *Trialeurodes vaporariorum*. The optimal schedules and compatible mixtures of pilot products with other PPPs essential for plant protection in the 3 crops (citrus, tomato/pepper, cucurbits) to be used in combination with existing biological control strategies are reported as well as possible side effects on beneficial arthropods.

This deliverable is part of an ongoing manuscript for a scientific peer-reviewed publication. It will be submitted to a scientific journal soon (likely submission date is 2023), and, once published (gold open access), will be linked to this deliverable. The details contained in this study are still confidential until online publication by the journal.

2 State of the Art of Essential Oils and Vibrations to control Whiteflies

The ecological impact of pesticides has been a growing concern since the second half of the 20th century. Yet, the use of pesticides has neither stopped nor decreased (Guedes et al., 2016). Rather, alternative technologies and pest management strategies have been explored and developed to overcome the issues of persistence, pest resistance and eco-toxicity; while maintaining ecological balance and preserving on-farm biodiversity (Butu et al., 2020; Ribeiro et al., 2021). One strategy has been to restrict the use of synthetic inputs and to adopt effective alternatives, namely by enhancing ecosystem services (i.e., biological control) and using environmentally friendly products such as mineral oils or biopesticides in the context of organic agriculture (Regnault-Roger et al., 2012; Shennan et al., 2017). Mineral oils are petroleum-based products well integrated into modern and sustainable practices given their low persistence and residual activity, and low risk of pest resistance (Buteler and Stadler, 2011). However, given their side effects on plant physiology (e.g., inhibition of photosynthesis) and their wide spectrum, mineral oils are considered contentious products (Katsoulas et al., 2018). On the other hand, biopesticides are living biological organisms and/or their bio-metabolites that are used for the purpose of killing or inhibiting pests. They include entomopathogens, plant derived substances and pheromones and offer the advantages of reduced toxicity, lower persistence and rapid degradation (El-Shafie, 2019; Butu et al., 2020). Considering the contentious status of Mineral oils, biopesticides can be considered a viable alternative. However, the impact of pesticides on either pests or beneficial insects (predator, parasitoids, and pollinators) is multifaceted and goes beyond the simple acute toxicity (Desneux et al., 2007; Guedes et al., 2016). Insecticides can indeed affect insect life traits in ways that often escape our understanding, with toxicant exposures causing severe repercussions on insect physiology (i.e., fecundity, fertility, longevity, sex ratio) and behavior (i.e., mobility, orientation, feeding and learning) (Desneux et al., 2007; Rugno et al., 2019; Fernandes et al., 2010; He et al., 2013; Calvo-Agudo et al., 2020; De Armas et al., 2020; Ricupero et al., 2020). Such treacherous and poorly known effects can undermine the efficacy of biological agents for pest control, making farmers opt for the more "reliable and consistent" pesticides, and hampering the transition toward organic agriculture.

The Greenhouse Whitefly (GW), *Trialeurodes vaporariorum*, is considered one of the most harmful and economically important insect pests in greenhouses worldwide. It can develop in huge populations on many different crops and cause both direct damages, by piercing leaves and stems, thus subtracting nutrients, and indirect damage, because of its ability to transmit viruses to plants. The GW infestation makes fruit unmarketable because of the abundant honeydew emission which allows the proliferation of sooty molds that interfere with photosynthesis, reduces plant transpiration and favor the presence of mites (Bird & Maramorosch, 1978). Synthetic insecticides (in conventional farming) and biocontrol agents are the principal control methods of GW (Gorman et al., 2002). Another option for IPM and organic farming is the use of essential oil extracts and biocontrol agents (Hoddle et al., 1998).

In our preliminary trials, we separately applied *Clitoria ternatea* extracts (BPA044I) and Orange Oil (030-S-1-D) to zucchini and tomato plants to assess their efficacy on different GW life stages. 030-S-1-D showed a significant effect against adults both on zucchini and tomato, while this was low against nymphs on tomato, but high on zucchini.

BPA044I showed a lower efficacy against adults on zucchini and tomato plants, and against nymphs on zucchini, whereas the efficacy was higher against nymphs on tomato. As for the eggs, none of the tested products shown a relevant efficacy. From these trials we concluded that (1) the most vulnerable stages are adults and nymphs, while the eggs appear to be more resistant; (2) the combined use of BPA044I and 030-S-1-D has the potential to extend the general efficacy against GW adults and nymphs both on tomato and zucchini.

As for the use of Disruptive Vibrational Signals (DVS), we preliminary studied the mating behavior and associated vibrational communication of GW (Fattoruso et al., 2021). We characterized all vibrational signals and defined their role in the mating communication, by creating a detailed ethogram. We defined the GW frequency pattern of communication. We concluded that vibrational signals mediate mating behaviour and are crucial elements of courtship to achieve mating and that a possible approach to interfere with mating could be the transmission of DVS, designed to cover the dominant frequency range (100-300 Hz) typical of the GW communication.

3 Part A: Combination of BPA044II – 030-S-1-D and Vibrational signals against the greenhouse whitefly *Trialeurodes vaporiaroum*

3.1 Aims of the Study

The aim of the study was to evaluate, in greenhouse conditions, the effectiveness of the DVS transmitted to the host plant to control the GW populations. We also tested the preparation of BPA044I and 030-S-1-D combined (Combo) together with DVS against GW. Two set of experiments were conducted: on tomatoes and on zucchini plants.

3.2 Materials and Methods

3.2.1 Disruptive Vibrational Signals

DVS were designed with the aim to cover the dominant frequency of the GW mating signals during courtship (Fig.1). They were transmitted through "Vibro-plates" (Fig. 2) which consisted of a square plate made of wood (side length: 20 cm, thickness: 1 cm) which were covered by a plastic layer. An electrically powered (12V) mini-shaker (model Tremos, CBC-Biogard) was placed in the center, under the plate and the disruptive vibrational signal was allowed by a microchip installed inside the mini-shaker. A laser vibrometer was used to ascertain that DVS amplitude on the plants was at least of 30 μm/s (range: 40-300 μm/s), which is considered sufficient to mask the GH signal.

3.2.2 Preparation of the combination of C. ternatea extracts and Orange Oil

BPA044I, the product based on *C. ternatea* extracts, is an emulsifiable concentrate highly soluble in water. The product can be easily diluted and spray on the crops with all the standard devices used for conventional pesticides (e.g., back sprayers, atomizers). Moreover, the proteins contained in the extract are stable overheat.

Orange oil product is formulated as micro-emulsion, is diluted in water, and applied to the crop with standard spray devices used for conventional pesticides. For the control of whiteflies in open-field and greenhouse the label dose rate suggested is 400 mL/hL.

The two products **are physically and chemically compatible**, when mixed together in tap water we did not observe residues, deposits, formation of phases or any other negative trait. BPA044I and Orange Oil were used at **20 and 4 mL/L**, respectively, as final concentration in the spray solution. Tap water was used to dilute the products and application was performed with a pressurized hand sprayer.

3.2.3 Experimental Design

Five treatments: 1) BPA044I + 030-S-1-D (Combo); 2) Combo + DVS (C+DVS); 3) DVS; 4) Pyrethroid insecticide (Decis Jet), as a positive control (2.5 mL/L); 5) Water, as a negative control. Each replication consists in a Bugdorm cage (mod. 4S2260, 24.5 x 24.5 x 63 cm) containing three potted plants. Four replications per treatment. Each product was sprayed directly to the plants, on both the leaf surfaces. Dosages: Combo: BPA044I: 20 mL/L + 030-S-1-D Orange oil: 4 mL/L; Decis Jet: 2,5 mL/L

3.2.4 Protocol

1. Around 500 specimens of GW adults were released in each cage. They are left on the plants for 24 hrs. Then the plants are removed from the plants, cleaned from the insects and inserted in new cages.

2. After 15-17 days from the infestation, the plants are treated according to the experimental design. As for the vibrational treated plants, this was the moment of the vibro-plate activation. Vibro-plates were kept turned on for the rest of the experiment, without any pause.

   a. **Tomato**: after 14 days from the treatments, 3 random leaves of similar size and age per cage were collected. This sampling was repeated once a week for a total of 8 sessions (7 for pre-adult stages (eggs, nymphs and pupae) and 1. The last one, for adults). The collected leaves were observed under stereoscopic microscope to count the number of eggs, nymphs and pupae. The adult collection was done at the 8th weeks.

   b. **Zucchini**: after 14 days from the treatments, 5 random leaves of similar size and age per cage were observed and nymphs, pupae and adults were counted. Unlike tomatoes, it was not possible to collect leaves from plants without risking compromising the infestation. In fact, the whiteflies tended to aggregate to few leaves, which are larger than in tomato plants. Therefore, being not feasible to observe the leaves from the microscope we made direct observations and divided the leaves in 4 categories: "0": no presence of GW; "1" less than 10 specimens; "2" less than 30 specimens; "3" more than 30 specimens; this sampling was repeated once a week for 5 weeks.

3.2.5 Statistics

All data analysis and plotting were performed with R (R Core Team, 2018) and ggplot2 (Wickham. 2016). In tomato trials, two non-parametric tests were used to compare the number of individuals, for each treatment and the control. For the number of eggs and immature stages (nymphs and pupae), the Friedman test for repeated measures with replicates followed by Wilcoxon pairwise, Bonferroni corrected, post hoc test was used, considering the data collected in the different sessions. For the number of adults at the final session, the Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni correction, was used. The same test was done for zucchini trials where instead we used the above-mentioned quantitative categories.

3.3 Results

3.3.1 Tomato

All treatments contributed to keep the number of eggs and immature stages (nymphs + pupae) at lower densities compared to negative control (water). The population fluctuations observed for the plants on which only water was applied were not present in treated plants (Fig. 3). The number of eggs present on plants subjected to the different treatments was comparable and significantly lower than the control ($X2=18.71$, $df=4$, $p<0.001$). For nymphs and pupae, the lowest number was observed on plants treated with Combo, C+DVS and Decis Jet (positive control), intermediate on those treated with DVS and maximum for the water ($X2=44.45$, $df=4$, $p<0.001$) (Fig. 4). The lowest number of adults recorded at the last session was associated to plants with the C+DVS treatment ($X2=10.46$, $df=4$, $p<0.05$) (Fig. 5).

3.3.2 Zucchini

With the exclusion of Decis Jet, the populations tended to decline during the experimental period. At the first check, the GW population of the water control was higher than all other treatments while later C+DVS and DVS showed a clear reduction and at the last sampling their populations were much lower than those associated with the other treatments (Fig. 6). In general, the lowest presence of individuals was associated to the two treatments with vibrations (C+DVS, DVS), both significantly different from the two controls (Decis Jet and water). Treatment Combo showed intermediate values of whitefly populations (Fig. 7).

3.4 Conclusions

Our results indicate that for both crops there was a significant effect of DVS, alone or in combination with essential oils, in reducing the population of *T. vaporariorum*. In particular, while in tomato C+DVS produced the most relevant effects, and also the Combo treatment showed a good performance in maintaining low the GW population, in zucchini we did not find a clear difference between C+DVS and DVS, while the Combo effect was significantly

lower. Our hypothesis is that the plant structure, the type of leaves (i.e., size, shape), the presence of hairs and other characteristics can play a crucial role and thus determining the outcome of a treatment. This is probably the case of zucchini trials, in which the use of pyrethroids as positive control did not induce any suppression of the pest population. On the contrary, the use of DVS seems to be less affected (but not absent) by the peculiar plant structure and in both tests, we observed important GW reductions that were more relevant in tomato, especially if used in combination with the essential oils. Our conclusion is that the adoption of a strategy to control whiteflies in greenhouse should be chosen according to the type of crop and that the use of vibrations should always be recommended to reinforce other means of control. In this regard, disruptive vibrations could be seen as synergists.

4 Part B: Evaluation of the impact of BPA0441I – 030-S-1-D on a predator and a parasitoid of Citrus spp. pests

4.1 Aim of the Study

The main goal of the present work was to characterize the lethal and sublethal effects of candidate products for the replacement of Mineral oil, BPA044I (CT) and 030-S-1-D (OEO) (alone and combined), along with pesticides commonly used in organic agriculture (Mineral oil, Azadirachtin, Spinosad, and Pyrethrin), on two natural enemies of citrus pests: *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), a parasitoid of *Planococcus citri* Risso, and the predator *Cryptolaemus montrouzieri* (Hemiptera: Coccinellidae).

4.2 Materials and Methods

4.2.1 Insects and Plants

A colony of *Planococcus citri* was established in December 2017 from adults and ovisacs collected from sweet orange plants in the premises of CIHEAM-Bari (Valenzano, Bari). The colony was maintained on butternut squashes (*Cucurbita moschata*) inside Plexiglas and mesh cages (30x25x30 cm) under controlled conditions (25°C). The squashes were replaced weekly. *Leptomastix dactylopii* and *Cryptolaemus montrouzieri* colonies were set in 2020 starting from individuals provided by Bioplanet (Bioplanet, Cesena, Italy). Both parasitoids and predators were reared on butternut squash infested with *P. citri* inside Plexiglas and mesh boxes (30x25x30 cm) under controlled conditions (25±3 °C, 60 % RH, 14/10 L/D). The colonies were supplemented with droplets of honey, which was replaced every two days. Cocooned pupae of *P. citri* were individually isolated from the rearing to obtain freshly emerged virgin adults of *L. dactylopii* to use in the trials. The isolated pupae were placed in 15 mL plastic tubes supplemented with droplets of honey and were daily checked for parasitoid emergence. Citrus plants were six

months-old sweet orange plants var. Madam Vinous grafted on potted citrus plants (using the chip budding technique). Plants were maintained in a greenhouse under controlled conditions (25 C°, 16L/8D) and daily watered.

4.2.2 Tests on *Leptomastix dactylopii*

Three trials were conducted to evaluate the effects of pesticides used in organic agriculture on *L. dactylopii*. In trials 1 and 2, the influence of the residuals of pesticides on the survival and behaviour of the parasitoid *L. dactylopii* was assessed. In trials 3 we evaluated the effect of the exposure to the under-screening compounds on parasitoid foraging behaviour.

Citrus plants (20-30 cm height) were sprayed with the compounds under-screening with a hand-sprayer at the maximum dose indicated in the label until run-off. The plants were then allowed drying up for 3 hours under laboratory conditions (25±3 C°, RH 60%) and then caged inside a plastic and mesh isolator, excluding the pot. Each trial started with the release of the insects inside the isolator (hereafter, the arena), in order to expose them to the pesticide residuals for 72h. The tested products were the following: (i) Mineral oil (MO, 20ml/L)), (ii) BPA044I (CT, 20ml/L); 030-S-1-D (OEO, 6ml/L); (iv) a combination of OEO+CT (6+20ml/L), (v) Spinosad (SP, 0.5ml/L), (vi) Azadirachtin (AZ, 1.5ml/L), and (vii) Pyrethrin (PYR, 1.5ml/L) Tap water was used as a negative control.

4.2.2.1 Trial 1: Lethal effect after exposure to residues

To assess the lethal effect of the tested products on *L. dactylopii*, five females and five males (virgin adults, 24 – 48 h old) were released into the arena, which was provided with drops of honey (not treated with the pesticides) as food source. The parasitoids' survival was recorded at 1, 3, 12, 24, 36, 48, 60, and 72 hours after their release. Five replicates were carried out per treatment (thus 50 total individuals per treatment, 25 males and 25 females). The test was conducted in a growth chamber under controlled conditions (25±3 C°, RH 60%, 14L/10D).

4.2.2.2 Trial 2: Sublethal effects: parasitization rate and sex ratio

The female parasitoids that survived the first trial were tested to assess if the pesticide residuals could affect their parasitization rate and/or the sex ratio of their offspring. After pesticide exposure, females *L. dactylopii* were singly released into an arena with a citrus plant infested with 20 third-instar *P. citri* nymphs 24h before the parasitoid release. After a week from the parasitoids' release, the parasitized *P. citri* nymphs were counted and singly isolated in vials provided with honey drops. The parasitized *P. citri* were kept in a growth chamber (25±3 C°, RH 60%, 14L/10D) until adult parasitoid emergence. We measured both emergence rate and sex ratio.

In addition, an olfactometer trial was carried out to assess the potential effect of the pesticide residues on the *L. dactylopii* foraging behaviour, namely the ability to recognize and locate a plant infested with *P. citri*. Prior to be tested in the olfactometer, each female parasitoid was confined in a tube with a male for 24 h to allow mating; tubes were regularly checked in order to ensure that only mated females were used. Thereafter, *L. dactylopii* females were

exposed to the pesticide residuals as described above. The infested plants were citrus plantlets (var. Madam Vinous) inoculated three days before the olfactometer trial with 50-60 third instar nymphs and adult females of P. *citri*. The olfactometer was Y-shaped, composed by a single glass stem (15 cm long and 55.6 mm internal diameter) connected to two lateral glass arms (10 cm) separated at a 75° angle on the horizontal plane. Each arm was connected through a 20-cm plastic tube to the apical six-leaf portion of the plants isolated inside a plastic bag (Cuki oven bags, 25x38cm). Air produced by an air pump and inflating the plastic bag passed first through activated charcoal filters, then through a 1 L flask containing one-third of distilled water. A flowmeter connected to the tube linking the plastic bag to the olfactometer arm continuously recorded the air stream, ensuring the air was constantly flowing at 0.5 L min-1 arm-1. The olfactometer was placed horizontally on a plane and illuminated by a white fluorescent light tube (700 lux), mounted ca. 1 m above the olfactometer, to avoid biases due to light differences. The essay was carried out in a fully dark noiseless room, with the fluorescent tube as the only light source, at 25 ± 1°C. Females were individually released in the olfactometer and their behaviour was observed for ten minutes. To analyse the female movements, the olfactometer was sectioned into five main locations: the entrance of the main stem (namely, the starting point), the main stem, the bifurcation between arms and stem, the arm leading to the infested plant and the arm leading to the non-infested plant. The measured parameters were: 1) the number of "reactive" females (i.e. females that crossed the second half of the main stem from the release point); 2) latency period (time required from release to travel across more than 50% of the main stem); 3) the number of females making a choice between the infested and the non-infested plant (i.e., females that crossed the limit of 1.5 cm from the entrance of the arm, leading either to the infested or non-infested plant); 4) the time required to make a choice from release (between the infested and the non-infested arm); 5) the initial choice (the first arm chosen); 6) the final choice (where the parasitoid was at the end of the 10 min observation period); 7) the occurrence of a change in the choice made by the parasitoid regarding the selected arm; 8) the number of times such switches occurred. Moreover, we took note of the movement patterns (transitions) of the parasitoids between olfactometer sections. In order to simplify the reading of transitions, loops were discarded, as were intermediary transitions that lasted less than 15 seconds (similar to the principle of transitivity: if a parasitoid goes from point A to B and directly from B to C, we note that it went from A to C). The proportions of occurrence of each transition were calculated based on the frequency of said transition divided by the total number of transitions with the same starting point and were summarized in graphs, allowing to visualize the overall variation in behaviour between treatments. After each observation, the arms were disconnected from the plastic bags and were attached directly to the charcoal filters allowing ambient air to flow for five minutes. Every five females tested, the olfactometer arms were flipped 180° to minimize positional bias. At the end of the trials, the olfactometer was cleaned with acetone and rinsed with distilled water. The day of the trial, 10 females were tested (5 exposed to tap water and 5 exposed to one of the treatments), while a minimum of 20 replicas per treatment was carried out.

4.2.2.3 Statistical analysis

All statistical analyses and graph elaboration were carried out using R (R Core Team, 2021). The lethal effect of pesticide residuals on adult parasitoids was evaluated through Kaplan-Meier survival analysis and Multivariate Cox proportional hazard model (packages survival and survminer (Kassambara et al., 2021; Therneau, 2021)). The effect of the exposure to pesticides residues on the parasitization rate and sex ratio was assessed via Kruskal-Wallis rank sum test followed by Dunn test post-hoc for pairwise comparison in case of significant differences. Given that some treatments were performed in different periods (see above), two different datasets were created. In case of significant differences between the datasets, the analyses were run separately, while in case of non-significant differences, the datasets were combined as one. The parasitoids response parameters were divided into binary and scale variables. The binary variables, namely whether (i) a parasitoid was reactive, (ii) made a choice and (iii) chose infested, were analysed using a generalized linear mixed model (glmer – "lme4" package (Bates et al., 2015)) with treatment as the explanatory factor and dates as a random factor. We assumed a binomial distribution for binary variables. The scale variables, namely time for parasitoids to be reactive and time to make a first choice, were log-transformed and then analysed via a linear mixed effects model (lme – "nlme" package (Pinheiro et al., 2021)). Due to a high number of non-available values (NA), the variables regarding the parasitoid final choice, the occurrence of choice change, and the number of times changes occurred were discarded.

4.2.3 Tests on *Cryptolaemus montruzieri*

We evaluated survival and predation of both larvae and adults of *C. montrouzieri*: i) for 24h on plants infested with treated *P. citri*; ii) for 72h on untreated infested citrus plants following the 24h exposure to the infested treated plants. Citrus plants (20-30 cm height) were sprayed with the compounds under-screening with a hand-sprayer at the maximum dose indicated in the label until run-off. The plants were then allowed drying up for 3 hours under laboratory conditions (25±3 C°, RH 60%) and then caged inside a plastic and mesh isolator, excluding the pot. The tested products were the following: (i) Mineral oil (MO, 20ml/L)), (ii) BPA044I (CT, 20ml/L); 030-S-1-D (OEO, 6ml/L); (iv) a combination of OEO+CT (6+20ml/L), (v) Spinosad (SP, 0.5ml/L), (vi) Azadirachtin (AZ, 1.5ml/L), and (vii) Pyrethrin (PYR, 1.5ml/L) Tap water was used as a negative control. Three days before the experiment onset, citrus plants were infested with 20 third instar nymphs of *P. citri* each. The infested plants were then sprayed with the tested products with a hand sprayer until run-off, and allowed to dry up for 3 hours under laboratory conditions (25±3 C°, RH 60%). Thereafter, each plant was caged inside a plastic and mesh isolator excluding the pot (Figure 11). A single 10-days old *C. montrouzieri* deprived of food for 24 hours was then introduced inside the cage. Predator survival and predation rate (number of nymphs consumed) were assessed for 24 hours following the individual release inside the isolator, with 20 replicates (10 males and 10 females) per treatment. At the end of the 24 hours, the predator was moved to an untreated infested citrus plant; daily consumption rate and survival were evaluated at 1, 3, 12, 24, 48 and 72 hours after insect release on the plant. The same test was repeated using third instar *C. montrouzieri* larvae.

4.2.3.1   Statistical analysis

The statistical analysis was performed with the software IBM SPPS version 26. The data from survival tests were elaborated using survival analysis Kaplan-Meier and Cox model. The consumption rate (counting) data were elaborated using Kruskal-Wallis and Dunn test.

4.3 Results

4.3.1 Tests on *Leptomastix dactylopii*

4.3.1.1   Trial 1: Lethal effect of exposure to residues

Among the tested compounds, Spinosad (z=10.797, p<0.0001) and Mineral oil (MO) (z=3.119, p=0.0018) were the two compounds inducing a significantly greater mortality compared to the other products tested. Spinosad caused 100% mortality within 12 hours, while Mineral oil resulted in a mortality four times higher than the control. In addition, females were twice more likely to be affected by MO compared to males. In the case of Mineral oil, males had 45% higher probabilities of surviving than females exposed to the same product. Moreover, the combination OEO+CT showed a marginally non-significant effect (z=1.733, p=0.08306), causing 12% mortality within 72 hours. Currently, more replicates are being carried out to confirm previous findings (Fig. 8).

4.3.1.2   Trial 2: Sublethal effects of exposure to residues

The parasitization trial revealed that the number of parasitized nymphs and the progeny's sex ratio were not influenced by the exposure to the treatment residuals. However, the olfactometer trial (2.4.2) revealed significant differences among the treatments, particularly in the parasitoids' reactivity. Indeed, the parasitoids exposed to CT showed a significantly higher latency (p= 0.0308) (time spent in the first/initial portion of the olfactometer, close to the release point) exceeding ten times the latency period of the control (Fig. 9). However, within reactive females, there was no significant differences in the choice of the olfactometer arms. The analysis of the behavioral transitions (see the ethograms, Fig. 11) is currently ongoing.

4.3.2 Tests on *Cryptolaemus montruzieri*

During the first 24 hours of exposure to the residuals of the products tested, the survival of *C. montrouzieri* adults was overall similar among the treatments. Once moved to untreated infested citrus plants for 72 hours, a mortality of ca 40% was observed for adult individuals previously exposed to the combination CT+OEO (z=1.680, p=0.092). Regarding the predation rate of *C. montrouzieri* adults during the 24 hours following the residuals exposure, CT and the combination CT+OEO affected the predation rate with no sex-related difference. The average consumption

rate was 5.57±0.6 third instar nymphs of P.citri on CT-treated plants (z= - 9.601, p=0.000) and 6±0.8 third instar nymphs (z=9.270, p=0.000) on CT+OEO treated plants, with both the values significantly lower than the control (18.43±0.42 3rd instar nymphs/24h). The statistical analyses showed no significant difference in the survival of C. *montrouzieri* larvae exposed to the different insecticides neither during the 24 h exposure (x2=4.783, p= 0.442), nor during the successive 72 h (Likelihood ratio test=4.75 on 5 df, p=0.4473). The predation of *C. montrouzieri* larvae was significantly impacted by exposure to preys treated with CT (z = - 9.316, p=0.000), and to a minor extent to MO (z = 5.482, p = 0.000), OEO+CT (z = 6.577, p = 0.000) and SP (z = 5.338, p = 0.000). The lowest amount of *P. citri* nymphs consumed during the 24h following the exposure to compounds residuals was observed for CT (2.68±0.17 nymphs consumed in 24 hours), followed by OEO+CT (4.48±0.26 nymphs), and MO and SP (5.14±0.28 and 5.28±0.35 nymphs respectively). Further tests are being carried out to consolidate the dataset. Additionally, a model is being developed to predict the long-term effect caused by the exposure to the tested product on the predator population under field conditions (Fig. 10).

5   Conclusions

Our results indicate that more research is needed to evaluate the compatibility of BPA044I (CT) and its combination with 030-S-1-D (OEO) with biological control agents. Indeed, while neither CT nor CT+OEO seem to be acutely toxic for the predator and the parasitoid tested, the observed effects other the mere acute toxicity raise concerns. CT might act as feeding deterrent against the ladybird, displaying an effect similar to the one we observed in previous experiments on the orange spiny whiteflies *Aleurocanthus spiniferus* (Mokrane et al. 2020). In addition, 24h exposure to the residuals of CT in combination with OEO might affect the predatory activity, with a significant decrease in the predation rate when the individuals were moved on untreated infested plants. The impact of CT residuals exposure on the parasitoid appear even more treacherous: the observed delay in responsiveness to the odours emitted by a plant infested with its host suggests CT might significantly affect the foraging behaviour, thus consequently the ecosystem services provided by the species. In contrast, OEO appear to be a safe alternative to mineral oils at least for the beneficials tested, with no evident lethal or sub-lethal effects observed neither for the parasitoid nor for the predator upon exposure to the residuals of the toxicant.

This deliverable is part of an ongoing manuscript for a scientific peer-reviewed publication. It will be submitted to a scientific journal soon (likely submission date is 2023), and, once published (gold open access), will be linked to this deliverable. The details contained in this study are still confidential until online publication by the journal.

6   References

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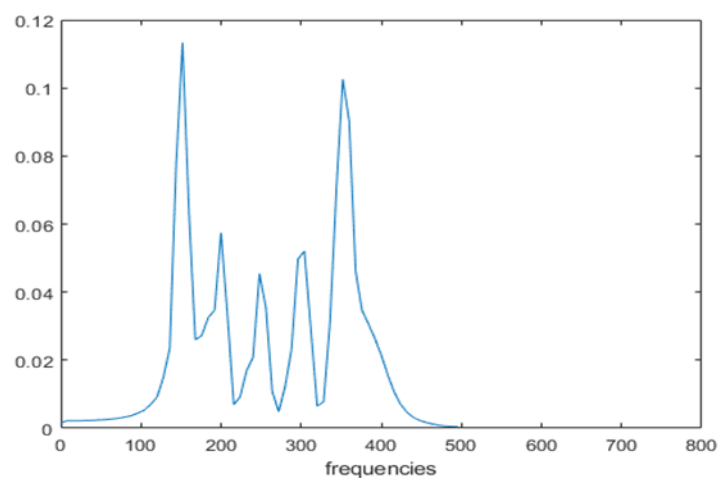
7 Figures

Figure 1: Sonogram of the Disruptive Vibrational Signals, used during the trials on tomato and zucchini in greenhouse. The signal frequency range was between 150-350 Hz.

Figure 2: Vibroplate. Under the centre of the square plate on which the plants are positioned, a mini-shaker Tremos (CBC-Europe) which emits the disruptive vibrational signals.
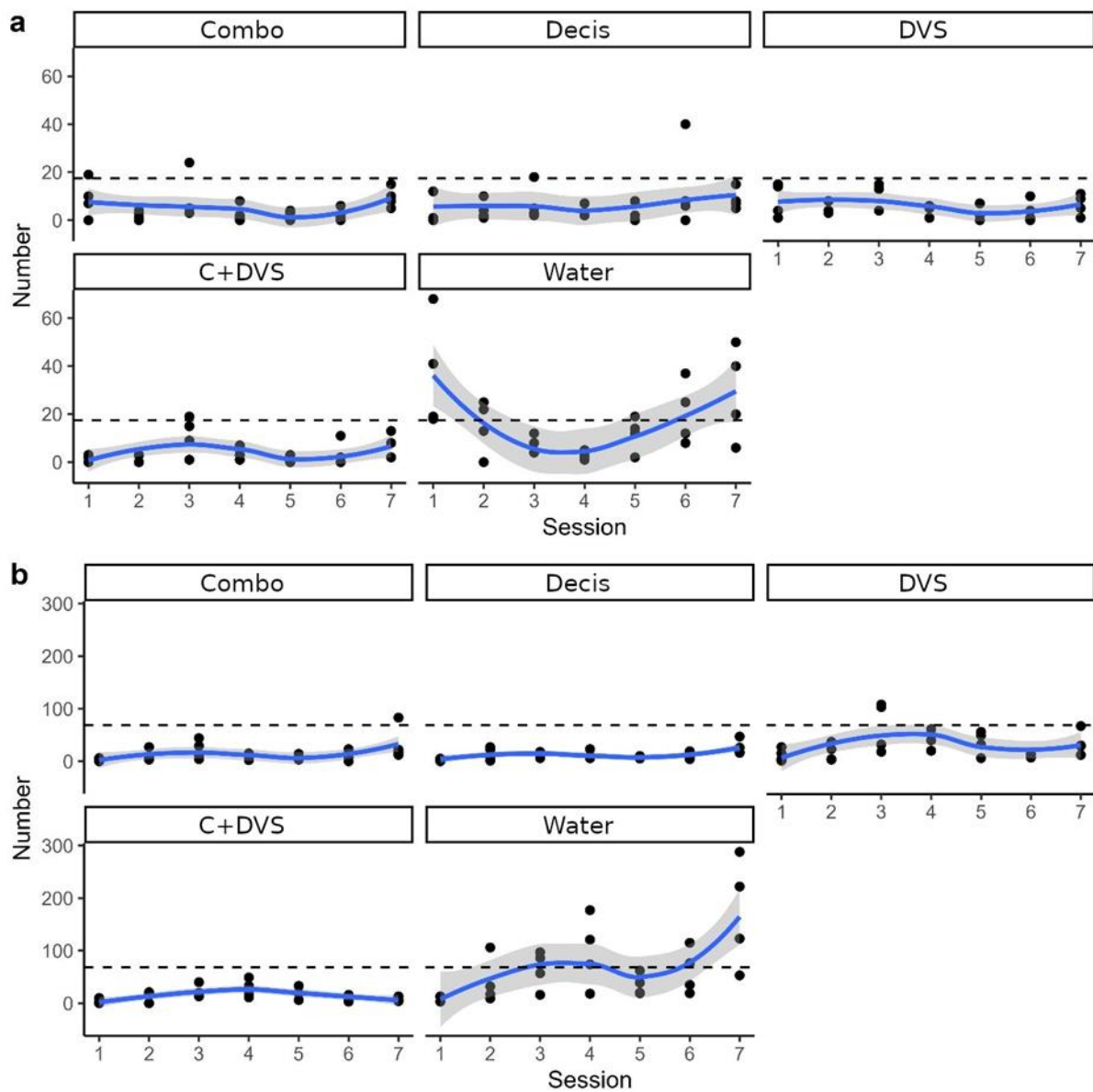
Figure 3: Number of recorded: (a) eggs and (b) nymphs and pupae of GW at each session, for the different treatments on tomato plants. Lines show the loess smoother, with 95% standard error confidence interval. Horizontal dashed lines show the average number for the negative control (water).
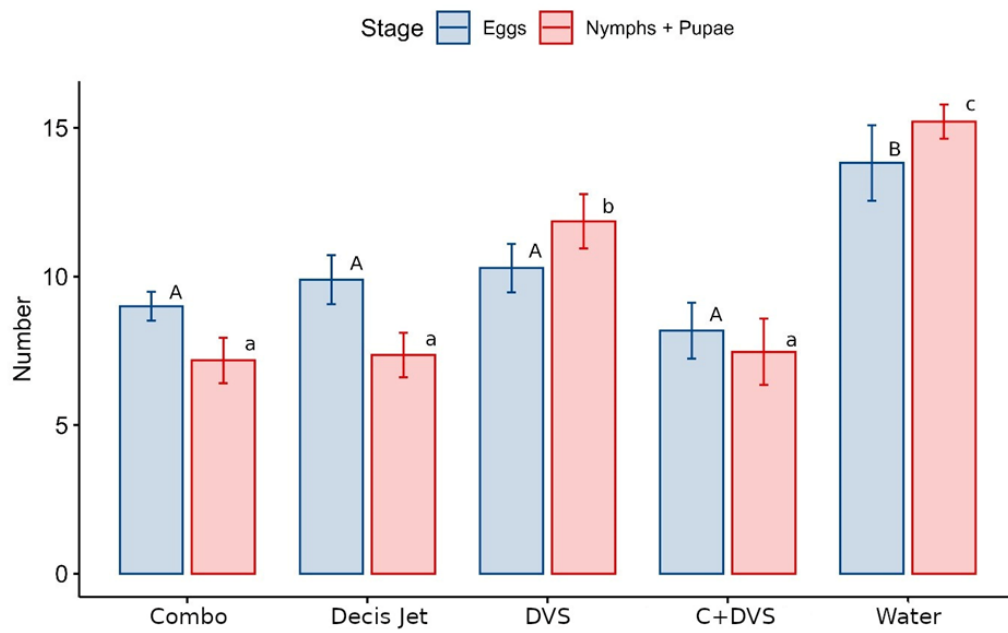
Figure 4: Barplot showing the average number (± SE) of GW individuals collected from tomato plants throughout the sampling sessions (1st-7th), according to their life stage and treatment. Different letters indicate significant differences (p < 0.05) after Friedman test with replicates: lowercase for eggs and uppercase for nymphs and pupae.
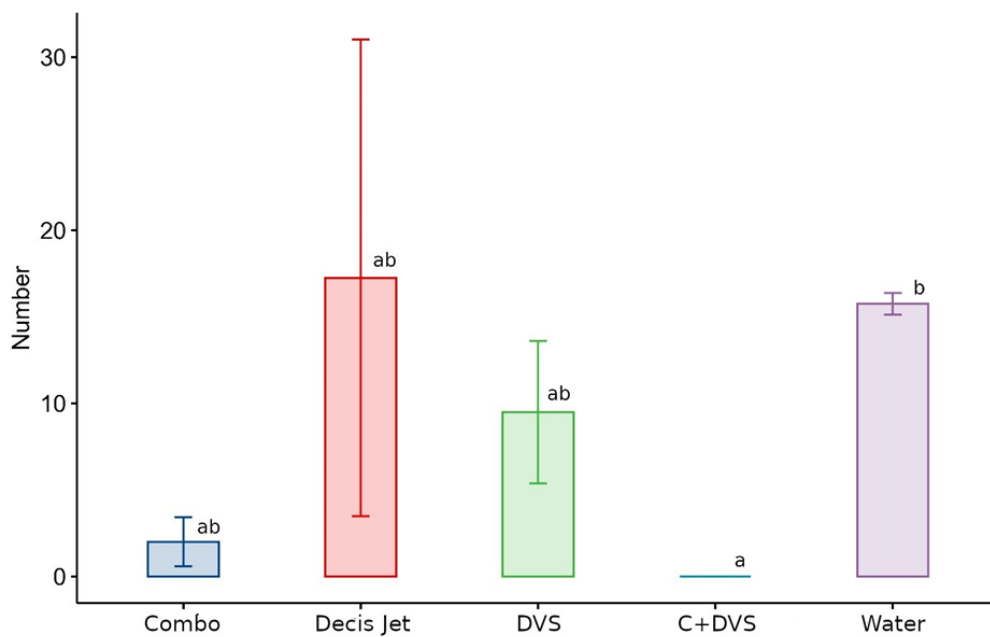
Figure 5: Barplot showing the average number (±SE) of GW adults on tomato plants, at the final (8th) session. Letters indicate significant differences (p < 0.05) after Dunn test, with Bonferroni correction.
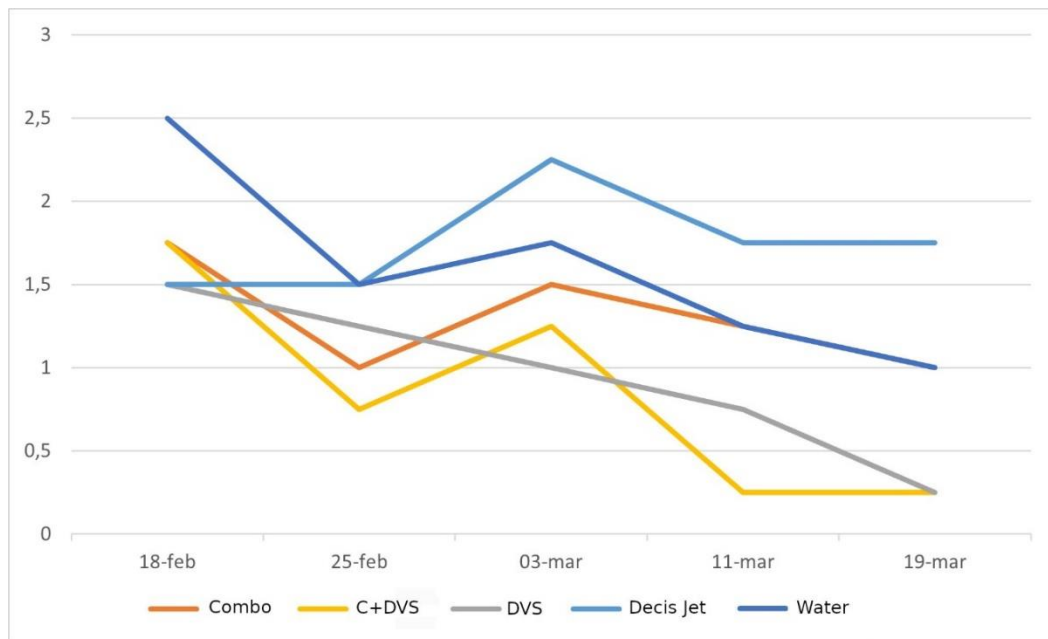
Figure 6: mean category of infestation per date of sampling of whiteflies (nymphs, pupae and adults) observed from 5 leaves of 4 zucchini plants treated with Combo, C+DVS and DVS. The positive and negative control were Decis Jet [4] and water [5], respectively

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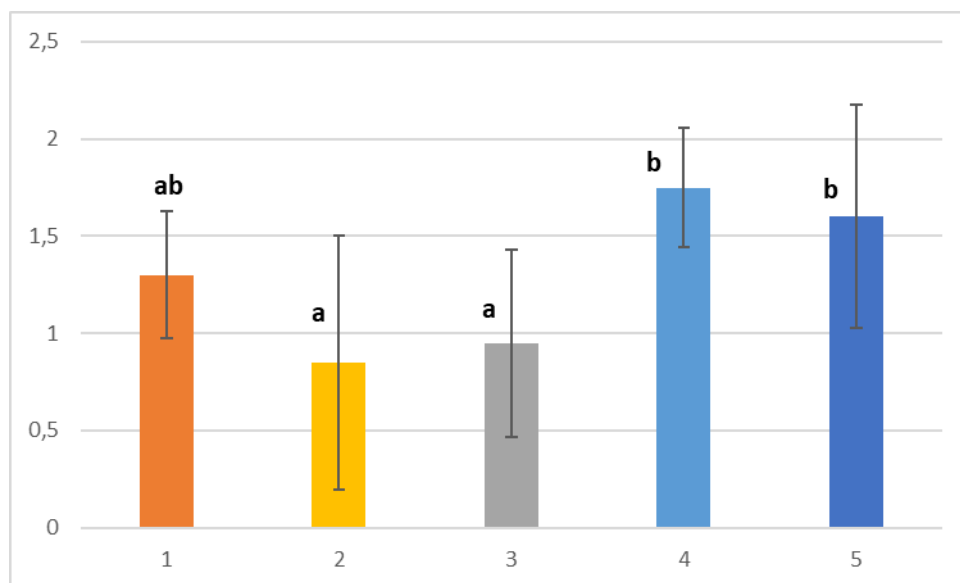
Figure 7: mean (±SD) quantitative category ("0"= no insects; "1" = less than 10; "2"= less than 30; "3" = more than 30) of whiteflies (nymphs, pupae and adults) collected from zucchini plants during the trial period per treatment: Combo [1], C+DVS [2] and DVS [3]. The positive and negative control were Decis Jet [4] and water [5], respectively. Different letters indicate significant differences after Friedman test followed by Wilcoxon pairwise post hoc test.
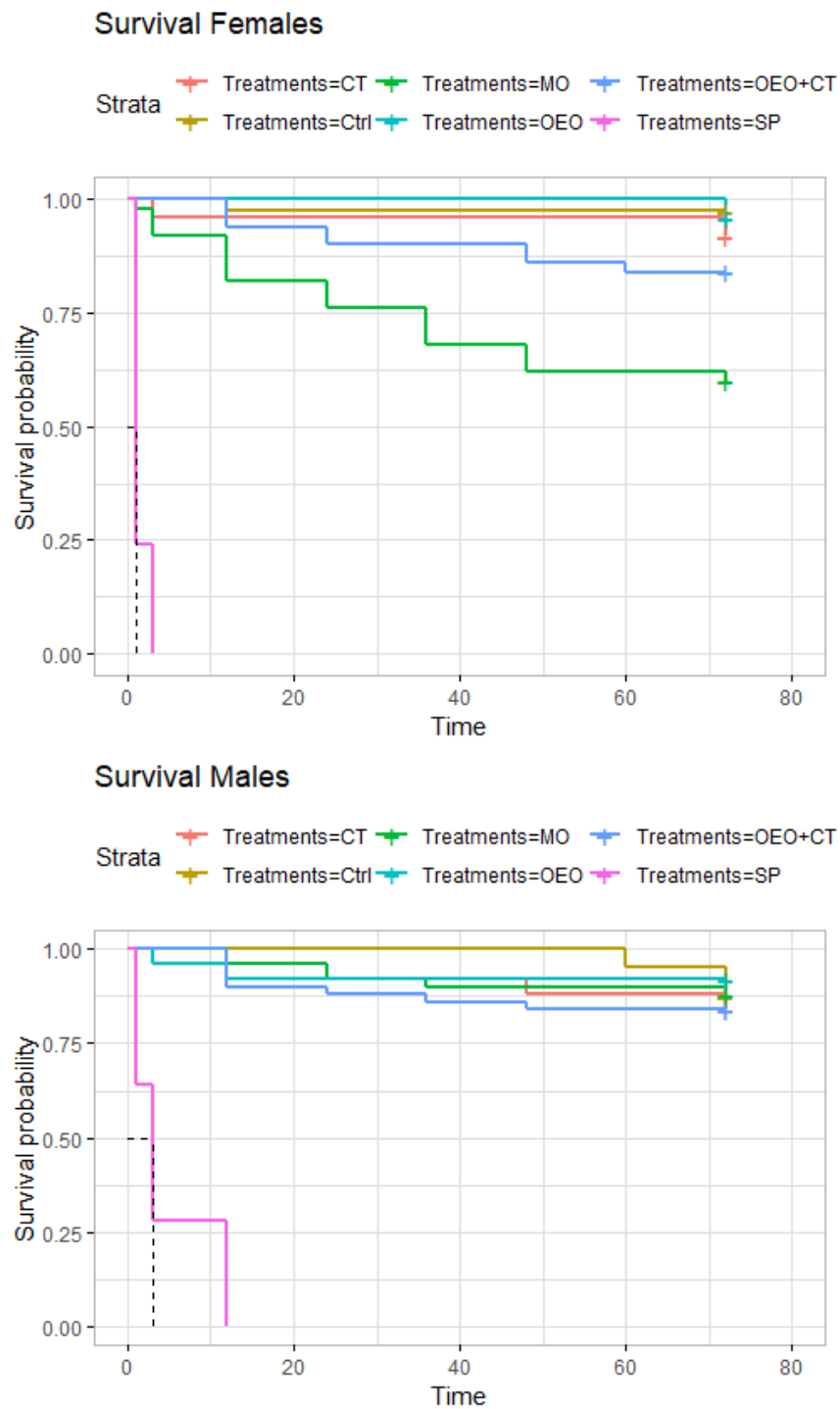
Figure 8: Survival curves of adult male and female Leptomastix dactylopii *during 72h exposure to insecticide residues*

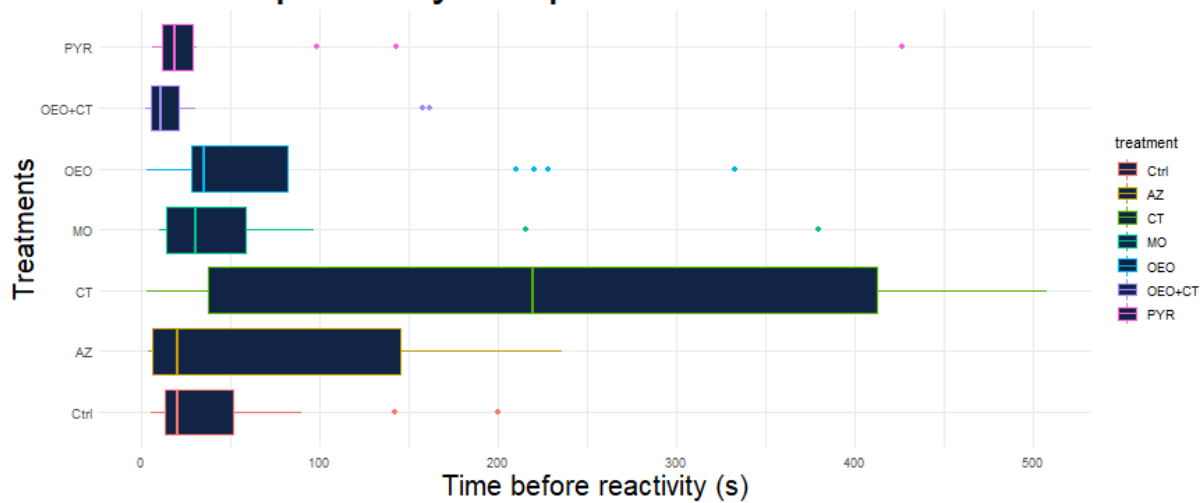# Time required by the parasitoids to be reactive

Figure 9: The effect of treatments on the latency of reactivity to the host in Leptomastix dactylopii *females*
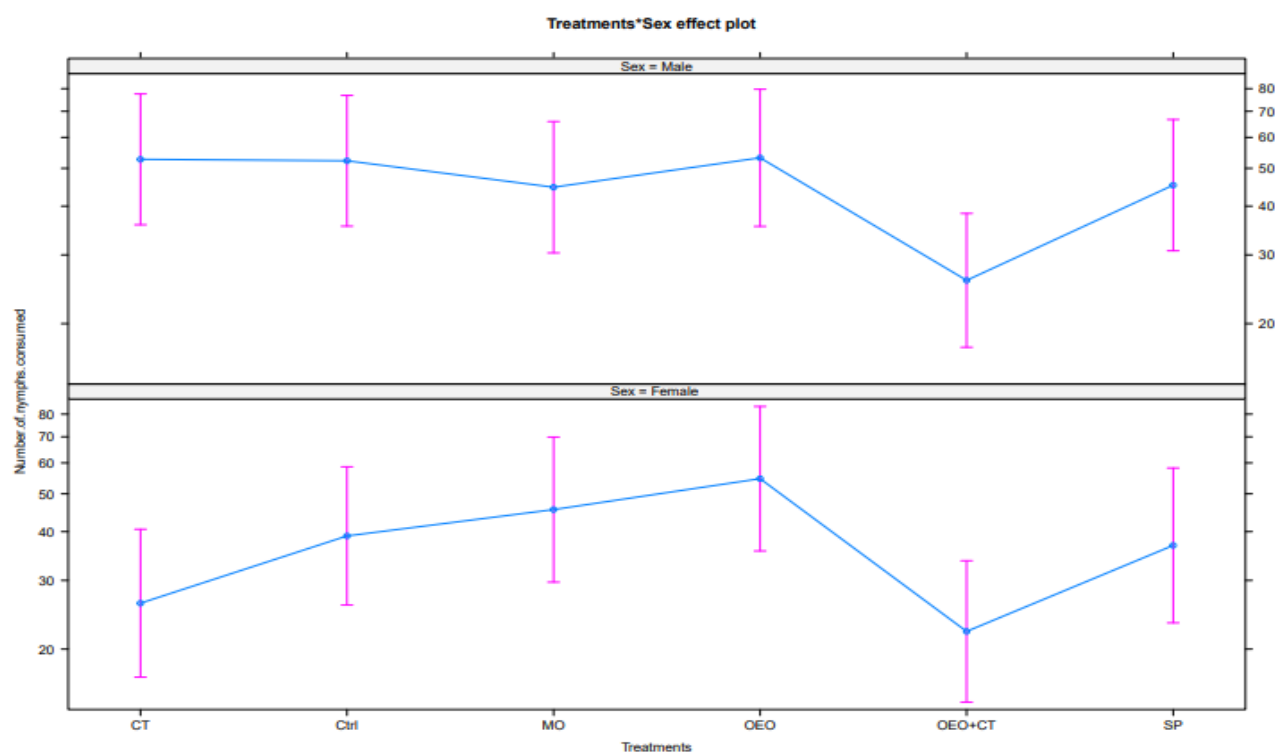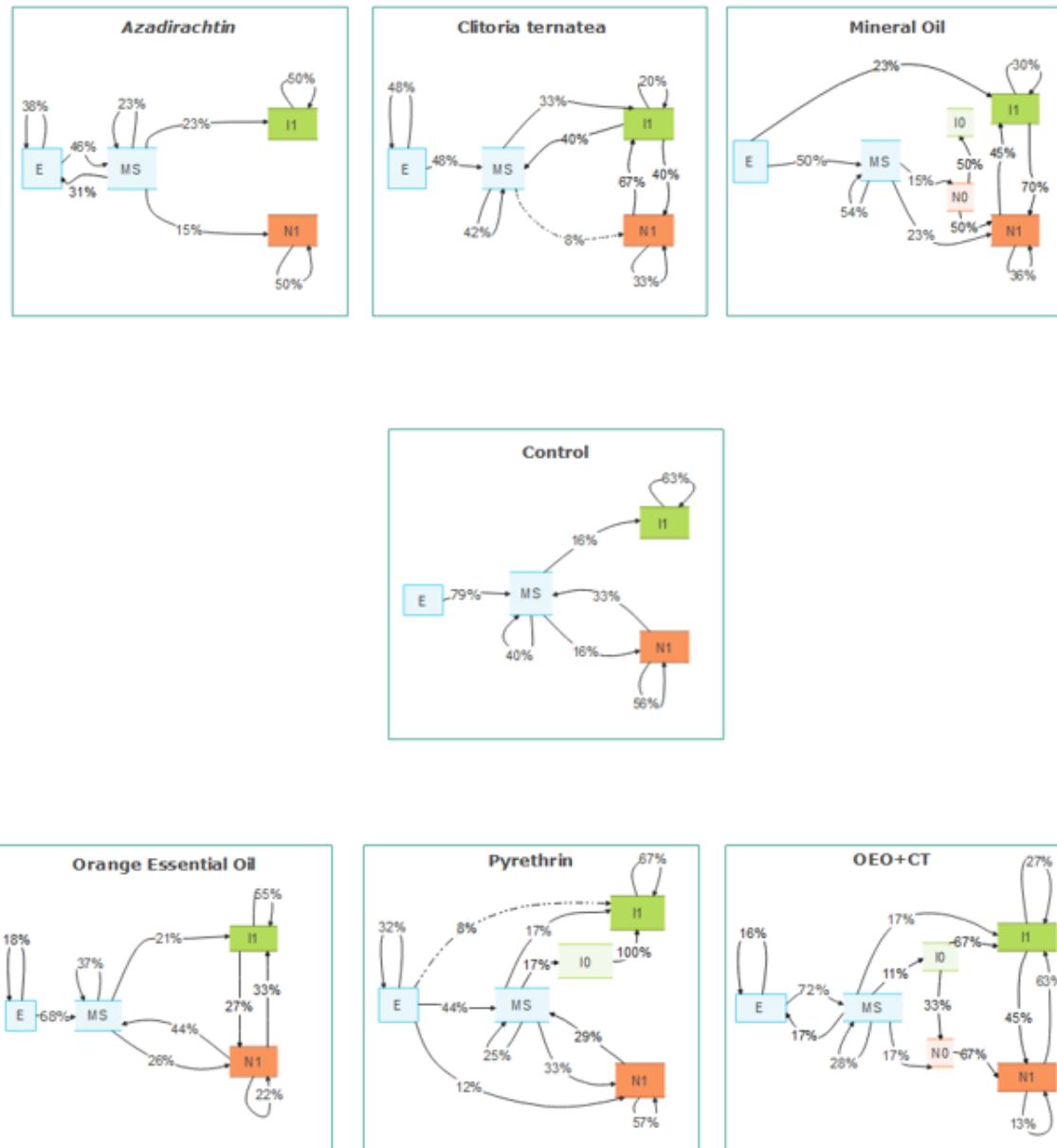

Figure 10: Predation of adults Cryptolaemus montrouzieri *males and females on* P. citri *nymphs during 72 hours following the exposure to residuals*

Figure 11: Ethograms, foraging behavior of Leptomastix dactylopii upon insecticide residuals exposure