



Impairing the development of an olive fly pest by targeting its symbiotic bacteria in egg-infested fruits

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With 5 figures

Abstract: The olive fly *Bactrocera oleae* is an important pest across the world, and its control has traditionally been achieved through synthetic insecticides. Nowadays, there is a growing demand for reducing chemical use due to environmental and human health concerns. The manipulation of symbiotic bacteria inhabiting insect pests has been proposed as a sustainable strategy, allowing insecticide-free management. Here, the possibility of impairing the strict association between the olive fly and its primary symbiont, ‘*Candidatus* Erwinia dadicola’, was tested by preventing bacterial infection of the newborn larvae of the pest. Five commercial products with putative antimicrobial activity were used for treating olives infested with eggs. Results showed a significant reduction of the symbiotic bacterial load in larvae derived from olives treated with dodine and a zinc-copper-citric acid biocomplex, as well as a reduction in the pupation rate of the pest. Our findings for the first time provide evidence of the feasibility of impairing the symbiotic association in olive fly through the application of antimicrobial compounds in olive fruit infested with newly laid eggs.

Keywords: *Bactrocera oleae*, Tephritidae, antimicrobial, primary endosymbiont, dodine, propolis, copper, zinc

1 Introduction

Olive tree groves cover more than 10 million hectares of land worldwide and in 2019 olive production exceeded 20 million tons per year (FAO 2019). The olive fly, *Bactrocera oleae* (Rossi 1790) (Diptera, Tephritidae) is the most destructive pest of olives and represents the major threat to the production (Commonwealth Institute of Entomology 1996). The female fly lays its eggs in the olive fruit, and the newly hatched larvae feed upon the pulp forming tunnels inside the drupe. This usually results in severe reductions in quality of olives, as well as in production loss due to fruit dropping throughout ripening (Daane & Johnson 2010). Moreover, olive production is threatened under favourable weather, since in these conditions the fly produces several generations per year and reaches extremely high population densities (Tzanakakis 2006). Traditionally, the control of *B. oleae* infestation has been successfully achieved through synthetic insecticides, often applied indiscriminately. The insecticides have, however, serious limitations due to their toxic effects on human health (Douglas 2007), the presence of residues in the olive fruit and olive oil (Bueno & Jones 2002; Kampouraki et al.

2018), the development of insect resistance (Kakani et al. 2010), and the negative impact on non-target arthropods and olive oil quality (Pinheiro et al. 2020). Moreover, the EU Regulation 2019/1090 recently banned the use of dimethoate (an organophosphate), the insecticide traditionally used for the control of olive fly infestations (Tognaccini et al. 2019).

In recent times, control strategies based on the elimination of obligate bacterial symbionts of insects (symbiotic control) have been proposed as a sustainable option allowing insecticide-free pest management (Arora & Douglas 2017; Bigiotti et al. 2019; Gonella et al. 2020; Sinno et al. 2020). Good targets for the application of these strategies are insect pests strictly associated with the symbiotic bacteria which are transmitted to the offspring following an extracellular route, with the bacteria undergoing an environmental phase prior to their acquisition by new-borns (Bigiotti et al. 2019; Gonella et al. 2020). Since the bacteria are present on the surface of the chorion, and this is one of the very few moments when symbionts are vulnerable because exposed to the surrounding environment, the egg stage offers a good opportunity for disrupting symbiont acquisition. The olive fly is associated with the primary symbiont ‘*Candidatus*

Erwinia dacicola which is acquired by newborn larvae from the egg surface (Capuzzo et al. 2005; Estes et al. 2009; Ben-Yosef et al. 2015). During oviposition, females smear the egg surface with bacteria and, upon hatching, the larva ingests the symbiont through nibbling the egg surface. The bacteria acquired during this period become associated with the alimentary tract and proliferate in the larval caeca (Capuzzo et al. 2005; Estes et al. 2009; Savio et al. 2012). The association between olive fly and the hereditary symbiont is essential for the fly lifespan in the harsh environment of unripe olives, since the symbiont detoxifies phenolic compounds (e.g., oleuropein) and is involved in the enzymatic hydrolysis of dietary proteins and amino acids synthesis (Ben-Yosef et al. 2015; Pavlidi et al. 2017; Estes et al. 2018).

Interrupting the symbiosis vertical transmission can also represent a promising strategy for *B. oleae* control in terms of both fly infestation reduction and environmental sustainability. Recently, some studies aimed to eliminate '*Ca. Erwinia dacicola*' and disrupt the vertical transmission targeting the adult stage of the olive fly (Bigiotti et al. 2019; Sinno et al. 2020).

It has been demonstrated that larvae without the symbiont show a drastic survival reduction in unripe olives (Ben-Yosef et al. 2015), whereas adult flies deprived of the symbiont have a strong loss in fitness and fecundity (Bigiotti et al. 2019; Sinno et al. 2020). Besides contributing to larval development and adult nutrition, the symbiont also affects the oviposition behaviour of *B. oleae* flies (Jose et al. 2019), with symbiotic and aposymbiotic flies differing in the number of oviposition attempts and oviposition preference between green and non-green olives.

This study aimed at disrupting the symbiont transmission from adults to larvae by directly treating eggs and infested olives with promising candidate antimicrobial products. Five commercial compounds were used in the experiments: copper oxychloride, dodine, wood distillate, flavonoids (propolis) and a micronutrient EC fertilizer namely Dentamet®. These products were chosen because they were already available in olive groves and presumably exhibit antimicrobial activity. The consequences at the larval stage in terms of life-history traits and symbiont load were studied to assess the feasibility of impairing the insect-bacteria symbiosis in-fruit at the earliest possible stage for a sustainable control of this major pest.

2 Materials and methods

2.1 Origin and rearing of flies

Wild pupae of olive fly were collected from a local oil mill in the Veneto Region (Northern Italy) in November 2021 and transferred to the laboratory within 24 h. To obtain cohorts of same-aged individuals, emerged flies were transferred into cubical net cages (side: 30 cm) and reared in a climatic

chamber INCOLD® (INCOLD SpA, Rovigo, Italy) at $23 \pm 2^\circ\text{C}$, $65 \pm 10\%$ RH, and 16:8 light:dark cycle.

Flies were fed a dry diet consisting of sucrose and yeast extract (Sigma-Aldrich) (4:1). Water was constantly available on a sponge wick and refreshed every 7 days.

Olives (cultivar "Leccino") were manually collected from a pesticide-free olive grove in north-eastern Italy (Bassano del Grappa – Veneto Region). Healthy olives were selected in the laboratory and then stored in open boxes at $6 \pm 1^\circ\text{C}$ until being used for rearing and experiments.

2.2 Exposure to the antimicrobial compounds

Among the commercial formulations already available for olive crops disease control or as plant biostimulants, five compounds known for their antimicrobial activity were chosen: copper oxychloride, dodine, tannins (i.e., wood distillate known to be also an elicitor, activating defensive plant metabolites, Kårlund et al. 2014), flavonoids (propolis), and a micronutrient EC fertilizer (zinc-copper-citric acid biocomplex, namely Dentamet®, hereafter Zn-Cu-citric acid biocomplex). The solutions were tested at label-recommended doses for their use in olive groves (300 g hL^{-1} , 165 mL hL^{-1} , 200 mL hL^{-1} , 300 mL hL^{-1} , 547 mL hL^{-1} , respectively) (Sup Table 1).

The experiments were performed targeting the primary symbiont smeared by the parent fly on the surface of the egg following two approaches: i) eggs directly exposed to antimicrobial compounds and ii) eggs indirectly exposed to the compounds by treating the previously infested olives with eggs laid in them.

2.3 Egg treatment

False olive fruits were made by melting agarose (1.5%) with a green pigment (PME Cake®, Powder colour, loose – olive green (2 g/0.07 oz) – code XPC307) in sterile conditions and cooling it in hemispherical silicone moulds (diameter 12 mm) at room temperature until solidification. The hemispheres obtained were then enveloped in Parafilm® to prevent them from drying out (Fig. 1).

To obtain olive fly eggs, the false olive fruits were exposed to mature flies (14 days-old) for 24 hours. Then, under a stereoscope, eggs were extracted one by one from the agarose false fruits using a scalpel, an awl and a small brush, then gently dipped into the solution for 10 seconds. Controls were represented by untreated eggs and eggs treated with distilled water. Furthermore, a treatment was conducted with a solution of sodium hypochlorite (NaClO 1%), considered to exert a high antimicrobial activity and deleterious effects on bacterial DNA on egg surface.

Subsequently, both treated and untreated eggs were dried by contact with absorbent paper. Eggs were carefully inserted into healthy olives where a hole had previously been drilled using an awl (1 mm diameter). One egg was placed into each olive. All operations were performed under micro-



Fig. 1. False olive fruits in agarose with green pigment (hemispheres) enveloped in Parafilm® used to obtain olive fly eggs.

biologically-controlled conditions in a laminar flow hood using a stereomicroscope and sterilized equipment.

For each treatment, an average of 40 artificially infested olives were prepared. The hole in the olive was then covered with parafilm to avoid dehydration. Olives were placed in plastic boxes and incubated at 20°C. After one week, olives were dissected, and the young larvae were immediately collected and counted. Olives reporting visible signs of rotting or moulds were not considered.

2.4 Olive treatment

To obtain infested fruits, healthy olives were exposed to mature flies (14 days-old) for a period between 12 and 24 hours. Olives showing at least 1–2 oviposition punctures were then selected under a stereomicroscope for the treatment. An average of 90 infested olives were randomly chosen per treatment and dipped for 10 seconds in the antimicrobial

solutions, then dried in a laminar flow hood at room temperature on absorbent paper. Controls were represented by untreated infested olives and infested olives treated with distilled water. Olives were kept separately according to the treatment and incubated at 20°C to allow eggs and larvae development. After one week, 30 olives for each treatment were dissected and dedicated to larvae sampling. The remaining olives (about 60 for each treatment) were kept at 20°C in rearing cages to allow larval pupation. Mature larvae exited from the olive were left to pupate on tissue paper placed in the base of the rearing cage. Then pupation rate was recorded.

2.5 DNA extraction and quantification of the symbiont load

Sampled larvae, derived from treated eggs and treated olives, were firstly rinsed with distilled-sterile water, then stored in absolute ethanol in 1.5 ml polypropylene conical tubes (Eppendorf) and preserved at –20°C until further processing. Before processing for DNA extraction, the ethanol was evaporated by leaving the tubes slightly open in a 55°C oven for approximately 12 hours.

To assess the level of symbiont load, total DNA was extracted from the whole sampled larva following the protocol described in Capuzzo et al. (2005) and eluted in 15 microlitres of distilled-sterile water. The abundance of the symbiont in larvae coming from different treatments was assessed by Real Time-PCR. The abundance of ‘*Ca. Erwinia dadicola*’ was estimated taking into consideration the amplification of the *recA* gene obtained with the primers previously validated in Livadaras et al. (2021): *recA-F* (5'-TCTACCGGTTTCGTTATCCCTG-3') and *recA-R* (5'-AACG ATAATCACGTCAACAGCAC-3').

To normalize data, the β -*actin* gene of *B. oleae* was used as housekeeping gene (*Act1F* 5'-CGGTATCCACG AAACCACAT-3' and *Act1R* 5'-ATTGTTGATGGAGCC AAAGC-3') (Livadaras et al. 2021). The DNA abundance related to β -*actin* allows normalizing the content of ‘*Ca. E. dadicola*’ in relation to the size of the larvae as well as to make up for the biases encountered due to any causal DNA loss during the extraction process.

Quantitative PCR experiments were conducted with PowerUp™SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Lithuania) in a 5 μ L total reaction volume, containing 1 μ L of diluted genomic DNA, 2.5 μ L of Master Mix and a solution of primers with a final concentration of 300 nM. The experiments were performed with a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, final extension at 72°C for 10 min. Reactions were followed by a quality control melting curve and terminated by cooling. Each sample was run in triplicate, and the threshold cycle (CT) of these technical replicates was averaged for each individual sampled.

For the estimation of the symbiont and insect DNA abundance, the $2^{-\Delta\Delta Ct}$ method was used as follows: the abundance of ‘*Ca. Erwinia dacicola*’ *recA* DNA was calculated relative to the *B. oleae* β -actin reference. The ΔCt between the reference gene (*B. oleae* β -actin segment) and the target gene (‘*Ca. Erwinia dacicola*’ *recA* segment) was calculated for each sample as $\Delta Ct = Ct \text{ target gene} - Ct \text{ reference gene}$. The $\Delta\Delta Ct$ between the ΔCt of the treated samples and the average ΔCt of the control groups was then calculated using $\Delta\Delta Ct = \Delta Ct \text{ treatment} - \text{mean } \Delta Ct \text{ control}$. Finally, the fold change $2^{-\Delta\Delta Ct}$ was calculated and averaged. Obtained data were log-transformed and the logarithm of the fold change $2^{-\Delta\Delta Ct}$ was used as an index of the relative abundance of the symbiont load in comparison with the control.

2.6 Statistical analyses

To evaluate the effect of egg and olive treatments on the relative bacterial load of ‘*Ca. Erwinia dacicola*’, the Dunnett’s test was used. In each experiment (egg and olive treatments), the abundance of ‘*Ca. Erwinia dacicola*’ was compared with the respective control.

To evaluate the effect of the treatments on pupation rate, a binomial model with a logit link-function was built and validated. The response variable was the pupation rate (number of larvae reaching the pupal stage divided by the number of olives: $n \text{ pupae} / n \text{ olives}$). The categorical explanatory variable was the treatment (seven levels). Pairwise multiple comparisons among groups were performed using post-hoc tests with Holm correction of p -values.

To evaluate the correlation between the bacterial load and pupation rate, a linear regression model was built and

validated. The response variable was the *Erwinia* load. The continuous explanatory variable was the pupation rate.

All statistical analyses were run in R software (R Core Team 2021).

3 Results

3.1 Effect of exposure of fly’s eggs to the antimicrobial compounds

The treatments exerted a negative effect on the symbiont ‘*Ca. Erwinia dacicola*’ load in larvae derived from treated eggs. The qPCR results showed that relative abundance of the symbiotic bacteria load resulted as significantly lower in all treatments compared to the control (non-treated) ($p < 0.05$). The strongest reduction in bacterial titre occurred in larvae hatched from eggs treated with sodium hypochlorite, followed by dodine and tannins treatments ($p < 0.001$) (Fig. 2).

3.2 Effect of exposure of infested olives to the antimicrobial compounds

The qPCR results showed a significant reduction of the relative abundance of the symbiotic bacteria ‘*Ca. Erwinia dacicola*’ in larvae derived from infested olives treated with dodine solution ($p = 0.009$) and the Zn-Cu-citric acid bio-complex ($p = 0.023$). No significant adverse effect was found in larvae from eggs treated with water, flavonoids (propolis), tannins or copper, even though the copper treatment led to a decrease of the symbiont titre (Fig. 3).

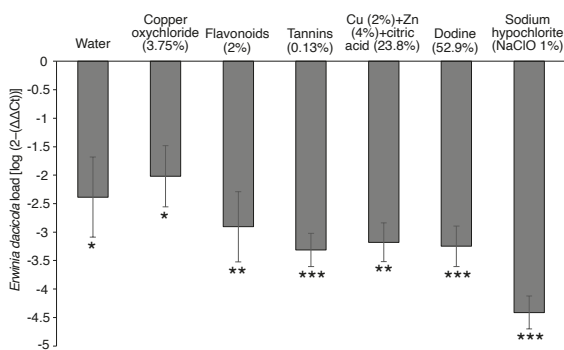


Fig. 2. *Candidatus* *Erwinia dacicola* load resulting from qPCR and expressed as mean value (\pm SE) of $\log(2^{-\Delta\Delta Ct})$ in the intestinal tract of *Bactrocera oleae* larvae hatched from eggs treated with the selected antimicrobial compound. The zero-level corresponds to the mean control load. The control consisted of larvae hatched from untreated eggs. Bars marked with an asterisk were significantly different from the control mean in Dunnett’s test (* = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$).

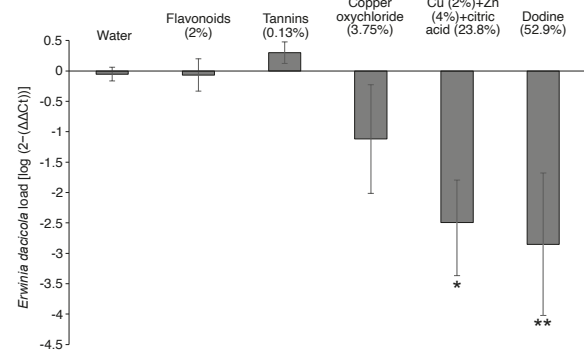


Fig. 3. *Candidatus* *Erwinia dacicola* load resulting from qPCR and expressed as mean value (\pm SE) of $\log(2^{-\Delta\Delta Ct})$ in the intestinal tract of *Bactrocera oleae* larvae hatched from infested olives treated with the selected antimicrobial compound. The zero-level corresponds to the mean control load. The control consisted of larvae hatched from untreated olives. Bars marked with an asterisk were significantly different from the control mean in Dunnett’s test (* = $p < 0.05$, ** = $p < 0.01$).

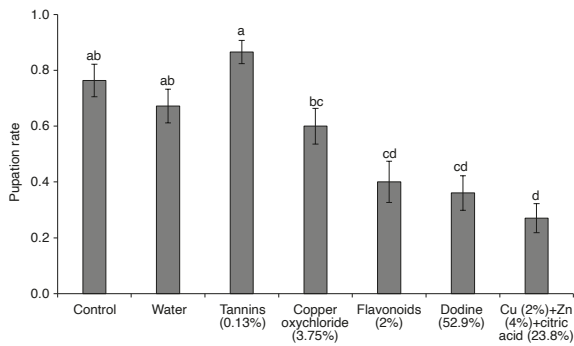


Fig. 4. Mean (\pm SE) pupation rate of *Bactrocera oleae* from infested olives treated with the selected antimicrobial compounds. Letters indicate significant differences ($p < 0.05$) in the pairwise comparisons between treatments (binomial model with a logit link-function).

3.3 Pupation rate and correlation with bacterial load

Taking into consideration the pupation rate, three compounds showed significantly lower values compared to the control ($p < 0.05$): Zn-Cu-citric acid biocomplex (Dentamet®), flavonoids and dodine (Fig. 4). Among these compounds, the application of Zn-Cu-citric acid biocomplex, showed the highest reduction of the pupation rate, followed by dodine, flavonoids (propolis).

A positive correlation was observed between the '*Ca. Erwinia dadicola*' load and the pupation rate of *B. oleae* (Fig. 5).

4 Discussion

The goal of targeting an obligate bacterial partner is to control insect pests by eliminating the microorganisms required for sustained insect development, reproduction and survival. This strategy involves the use of specific symbiocides, i.e., effectors that perturb the resident microbial partners and their interactions with the insect (Arora & Douglas 2017).

In this study, the feasibility of hampering the vertical transmission of the olive fly primary symbiont (*Ca. E. dadicola*) targeting the egg stage of the pest in infested olives was demonstrated for the first time. A significant reduction of the symbiont titre in the larval stage was observed after treating eggs and infested olives with antimicrobial products. These results indicate that the tested compounds were able to kill or reduce the bacterial load on the egg surface before being ingested by the new-born larvae during hatching, hence hindering the symbiont acquisition.

All tested products displayed an antimicrobial activity when treating the eggs directly, but only some performed their activity in infested olives. Among the products applied on the egg surface, even the water exerted a significant nega-

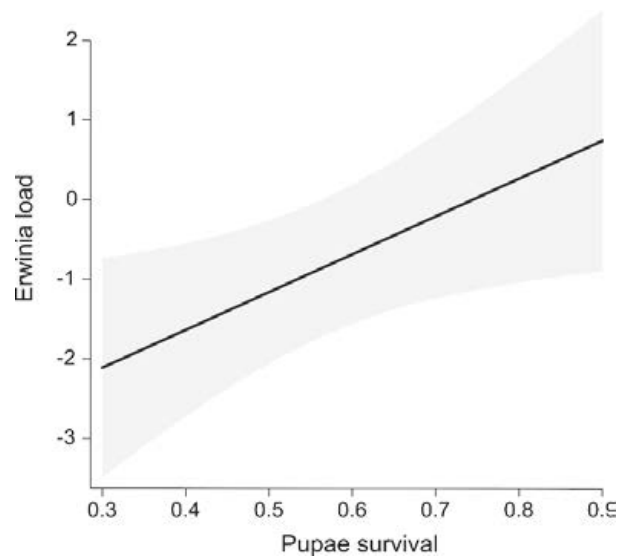


Fig. 5. *Candidatus Erwinia dadicola* load in relation to pupation rate from infested olives treated with antimicrobials. Plots include model estimate (black line) and 95% confidence interval (grey shading) from the linear regression model. Regression line equation: $y = 4.755x - 3.539$; $R^2 = 0.636$.

tive effect on the bacterial titre. It can be hypothesised that the direct application of water or other liquids could have washed away the symbiotic bacteria located on the egg surface, actively contributing to the decrease in symbiont load. Sacchetti et al. (2019), in a study on the importance of symbiont maintenance in *B. oleae* mass rearing, observed similar results when treating olive fly eggs with propionic acid, a mixture of sodium hypochlorite and Triton X, and water. Furthermore, considerable amounts of the *B. oleae* symbiont were retrieved in the rinsed water after washing the olive fruit. Regarding the treatments on infested olives, applications of the Zn-Cu-citric acid biocomplex (Dentamet®) and dodine showed the strongest and most significant effect against the symbiotic bacteria. Since the egg is laid inside the fruit pulp, right beneath olive epicarp (Malheiro et al. 2015), these two compounds could have reached the egg chorion by overstepping the physical barrier of the fruit epidermis or by capillarity through the oviposition puncture. In the latter case, the time elapsed between oviposition and the treatment application can be crucial to the success of the antimicrobials. After a few days, the oviposition puncture could suberify and close, reducing the ability of the compounds to reach the egg chorion.

Furthermore, a significant positive correlation between *Ca. E. dadicola* presence and *B. oleae* pupation rate was highlighted in this study. Larvae developed with a low symbiont load did not perform as control ones. Beside the reduction in the symbiont load, treatments with dodine and Zn-Cu-citric acid biocomplex exhibited a reduction in the pupation rate.

Since *Ca. E. dadicola* is essential for *B. oleae*, especially during larval development in the unripe olive (Ben-Yosef et al. 2015), the pupation rate reduction could have been influenced by the absence of the symbiont. A similar significant suppressive effect of Zn-Cu-citric acid biocomplex (Dentamet®) against the symbiotic bacteria *Pantoea carbekii*, and a higher mortality in its host nymphs was observed when treating eggs of the brown marmorated stink bug, *Halyomorpha halys* (Gonella et al. 2019). The Zn-Cu-citric acid biocomplex, Dentamet® is a micronutrient EC fertilizer, suitable for organic farming, that can be used in several crops such as olive trees. It includes zinc, copper and citric acid, which are known for their antimicrobial activity (Gonella et al. 2019). In olive groves, treatments with the Zn-Cu-citric acid biocomplex were demonstrated to inhibit growth of the pathogen *Xylella fastidiosa*, reducing the severity of symptoms (Scortichini et al. 2018). In particular, several studies reported the antibacterial activity of zinc against Gram negative bacteria including Enterobacteriaceae (e.g., Navarrete et al. 2014; Lallo da Silva et al. 2022). Likewise, citric acid has been demonstrated to display broad-range bactericidal activity (Tomotake et al. 2006). The negative effect exerted by copper against bacteria is also recognized and particularly, recent studies highlighted the symbionticide effect of copper on adults of *B. oleae* (Bigiotti et al. 2019; Sinno et al. 2020). However, in our study a lethal effect against *Ca. E. dadicola* was observed only when copper was used in combination with zinc and citric acid, whereas treatment with the single element did not decrease the symbiont load in a significant way. Similarly, the use of copper alone showed limited effects on the symbiont when treating eggs of *H. halys* (Gonella et al. 2019).

Dodine is a cytotropic-translaminal fungicide used in olive protection against *Spilocaea oleaginea*, the causal agent of olive leaf spot (Adawi et al. 2022). Beyond the antifungal action, it has demonstrated an antibacterial activity against some Gammaproteobacteria (the class including *Ca. E. dadicola*) (Cabral 1992). Also, other fungicides can exert an antimicrobial effect, such as Viridiol, which exhibited a negative effect against *Ca. E. dadicola* harboured in the adult olive fly (Sinno et al. 2020). The efficiency of dodine in reducing the symbiont load could be favoured by its cytotropic activity that allows the compound to reach the eggs laid inside the fruit near to the epidermis, with greater ease.

Treatments with flavonoids (propolis) showed a scarce effect on the reduction of symbiont load and a significant negative effect on pupation rate. In a previous study, *B. oleae* flies fed on a diet with flavonoids showed a high mortality of the adults but it was not associated with a reduction in their symbiont load (Bigiotti et al. 2019). As in our study, treatments with flavonoids exerted a negative effect on the insect host while no effect was observed on the symbiont load.

The significant negative effects of dodine and the Zn-Cu-citric acid biocomplex (Dentamet®) on both the bacterial

load and pupation rate of the olive fly, makes them interesting compounds for the promising strategy of Symbiotic Control (SC). Furthermore, in a recent study, dodine and the Zn-Cu-citric acid biocomplex showed a significant oviposition deterrent activity in treated olives (Checchia et al. 2022).

These products do not exhibit a direct insecticidal activity, and then no effects on the survival of beneficial insects should be expected. Orrù et al. (2021) tested the effect of the Zn-Cu-citric acid biocomplex (Dentamet®) on parasitoids of *H. halys* eggs and no evident negative impact on the fitness of these wasp species was found in short-term. Nevertheless, studies focused on the effect of these antimicrobial compounds on the non-target fauna in olive groves deserve further investigations. In addition, studies regarding the impact of these antimicrobials on the epiphytic bacteria of the olive tree will be useful for understanding possible effects of these treatments.

Symbiosis disruption has been successfully applied in insects such as stink bugs whose symbiotic bacteria, during the vertical transmission process, live on extrachorion secretions on the egg surface exposed to the surrounding environment (e.g., Taylor et al. 2017; Prado & Almeida 2009b; Gonella et al. 2019). Unlike these insects, *B. oleae* lays its eggs inside the fruit, protecting the eggs and symbionts from external threats. In this context, the efficiency of the antimicrobials could be questioned. However, our results demonstrate for the first time the possibility of hindering the vertical symbiont transmission of the olive fly by treating the olives with antimicrobials. In the light of these findings, these antimicrobial products can be considered important candidates for semi-field and field trials. There are, however, several variables that must be considered in the open field for an effective action of the symbionticide products. Time of exposure, stability of the products, volume, speed, pressure, and number of applications are crucial variables in this context. Among them, timing is particularly important, since treatments should be conducted when fruits are more susceptible to olive flies' oviposition and when the eggs are laid but not yet hatched. Young larvae deprived of bacterial assistance would be unable to feed on green olives and would die due to the effect of several antagonistic polyphenols (e.g., oleuropein) contained in the unripe olives (Ben Yosef et al. 2015; Bigiotti et al. 2019). Treating olives in this phenological stage could result in a synergic effect with the plants' antagonistic compounds for the fitness decay of larvae deprived of the symbiont. Furthermore, the decline of the insect's fitness makes it vulnerable to environmental stresses and to low impact control strategies against the pest.

Overall, our results open up clear future perspectives on the possibility of controlling the olive fly through symbiont control strategies, targeting the eggs inside the fruits. These methodologies could be also extended to other pests strictly associated with symbiotic bacteria.

Acknowledgements: This paper is dedicated to Professor Vincenzo Girolami and his long scientific activity. This research was supported by DAFNAE University of Padova through project BIRD_205285/20 “Individuazione di tecniche e strategie ecocompatibili per il contenimento della mosca dell’olivo *Bactrocera oleae* (Rossi)”.

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Manuscript received: 29 November 2022

Revisions requested: 10 February 2023

Modified version received: 15 June 2023

Accepted: 23 June 2023

The pdf version (Adobe JavaScript must be enabled) of this paper includes an electronic supplement: **Supplementary Table 1**