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Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*

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Abstract: Plant-extracted essential oils are generally suggested as potential sources for alternatives to synthetic insecticides in insect pest control strategies. The increased interest in the use of essential oils derives from the generalized perception of their safety for the environment, human health, and non-target organisms as well as a lower risk of resistance development. However, studies on essential oils have largely focused on their activity on targeted insect pests while overlooking their potential unintended effects on insect biological and reproductive traits, especially with sublethal exposures. Here, we first determined the toxicity of *Eucalyptus globulus* essential oil to adults of *Drosophila suzukii* and assessed the effects of low concentrations (i.e., LC_5 and LC_{20}) in old (5–7 days) and mated flies. Subsequently, we assessed longevity and fecundity in newly emerged virgin flies from four couples' combinations: unexposed couples, exposed females, exposed males, and exposed couples to the low concentration LC_{20}. Our results show that eucalyptus essential oil has good insecticidal activity against adults of *D. suzukii*. However, compared to untreated flies, the exposure to low concentrations enhanced the females' fecundity only when both old and mated female and male flies were exposed, while the females' but not males' life span was extended only in couples where newly emerged virgin females were exposed. Our findings suggest that although the eucalyptus essential oil may be a good control alternative for adult *D. suzukii*, its age-, sex-, and mating status-dependent stimulatory responses mediated by exposure to low concentrations need to be considered and further investigated.

Keywords: essential oils; spotted wing drosophila; toxicity; sublethal; hormesis

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1. Introduction

In the past decades, plants have been extensively screened for active compounds with potential practical applications [1–4]. Essential oils (EOs), as plant-derived extracts, are complex natural substances produced as secondary metabolites that have been scrutinized for their biological activities and are frequently proposed as a suitable alternative for controlling insect pests [3]. However, most of the investigations on plant EOs are carried out under laboratory conditions and are biased toward assessing their lethal and repellent activities against different insect pests and disease vectors. Consequently, potential ecotoxicological risks and non-target impacts of these compounds as well as the physiological and biological alterations in the targeted insect pests deriving from sublethal exposure to EOs have been frequently overlooked [2].

From a pest management perspective, the use of insecticidal products is still an important tool to reduce pest populations. Thus, most insects are repeatedly exposed to insecticide-mediated stresses that may result from exposure to low doses due to residue degradation [2,5]. Such exposure can affect different aspects of the exposed insects' behavioral, biological, and reproductive traits, and when it leads to stimulatory responses, it is termed as insecticide-induced hormesis. Hormesis is a biphasic dose–response relationship where a stressor that is toxic at high doses exhibits stimulatory effects at low doses in pests [5–7]. Beneficial and stimulatory responses resulting from exposure to low insecticide doses were described for different insect life traits including development time, longevity, fertility, fecundity, immune responses, locomotion, sexual communication, and feeding [5–7]. Based on their structure and associated physico-chemical and toxicological properties, EOs will act as any other insecticide, although they are generally natural mixtures of several molecules with many different modes of actions. Similar to synthetic insecticides, EOs were recently shown to induce hormetic-like responses in sublethally exposed insects [2,8–11].

The spotted wing fly *Drosophila suzukii* (Diptera: Drosophilidae) is an exotic pest with great economic importance capable of infesting a wide variety of hosts encompassing cultivated and wild red fruit species [12–14]. It is a native species from East Asia and was first reported in Brazil in the summer of 2012/2013 in the southern region of the country [15] before expanding its range to other regions of the country [16]. Management of this pest is performed mainly by the use of synthetic insecticides. To overcome the potential disadvantages of chemical control such as toxicity to non-target organisms and relatively high cost, the use of EOs has been suggested and investigated in *D. suzukii* control [17–23].

Eucalyptus is an Australian native plant, which is currently cultivated in subtropical and Mediterranean regions [24]. Among other multipurpose uses, essential oils extracted from the leaves of eucalyptus plants, like the Tasmanian blue gum *Eucalyptus globulus* Labill (Myrtales: myrtaceae), are reported to have a wide range of bioactivities including antioxidant, antimicrobial, fungicidal, antibacterial, acaricidal, nematicidal, and insecticidal effects [25]. Such biological activities are frequently attributed to the presence of compounds such as α-pinene, β-pinene, limonene, and mostly to the presence of Eucalyptol (1,8-cineole), which generally represents more than 70% (v/v) of the eucalyptus EOs [25,26]. Recently, a large number of natural extracts and compounds, including eucalyptus EOs, have been tested against *D. suzukii* [18]. However, the potential of sublethal effects mediated by plant-derived substances in this fly has been largely neglected in all the previous assessments of EOs' bioactivities.

Thus, in this research we used the spotted wing fly as the model to evaluate not only the lethal dose-induced but also low dose-induced effects of *E. globulus* essential oil. We documented stimulatory response in the longevity and fecundity in old mated as well as in newly emerged virgin flies.

2. Material and Methods

2.1. *Drosophila suzukii* Rearing

The stock colony of *D. suzukii*, used in the experiments, was kept in a rearing facility in the laboratory of Molecular Entomology and Eco-Toxicology (MEET) of the Entomology Department at the Federal University of Lavras, Lavras (Minas Gerais, Brazil). The flies were maintained in plastic cages (10 cm height × 25 cm diameter) using an artificial diet and following previously described methods [27,28]. The cages were kept under controlled conditions of temperature (T: 23 ± 2 °C), relative humidity (RH: 60 ± 5%) and scotophase (12H).

2.2. Eucalyptus Essential Oil

The EO of *E. globulus* was purchased commercially in sealed amber bottles with a volume of 10 mL from WNF Indústria e Comercio Ltd.a (São Paulo, Brazil) [29]. The major components are 1,8-cineole (94.4%), α-pinene (0.97%), β-pinene (0.33%), β-myrcene (0.4%), p-cymene (1.8%), trans-β-ocymene (0.55%), cis-β-ocymene (0.11%), and 1.47% of unidentified compounds.

2.3. Toxicity Assessment of Eucalyptus EO against *Drosophila suzukii*

The toxicity of eucalyptus EO was evaluated using the exposure method described in the protocol No.26 of Insecticide Resistance Action Committee (IRAC) with slight modifications. Briefly, pre-tests with logarithmically spaced concentrations of EO were carried out to determine the range of concentrations causing between 0 and 100% mortality. Once that mortality range was obtained, nine concentrations within it were used to determine the dose–response curve of the EO. The serial concentrations were prepared by diluting the corresponding EO volumes to the final concentration with dimethyl sulfoxide DMSO (2.5%) and a 20% sugar water solution. Then, dental cotton rolls (2 cm) were impregnated with 2.2 mL of the prepared serial dilutions of the EO and placed in 200-mL glass flasks. As a negative control, 2.5% DMSO in sugar water (20%) solution was used. Subsequently, for each repetition, 20 to 25 non-sexed flies of the same age (5–7 days) were introduced into each glass flask. Four repetitions for each concentration were used. The flasks were closed with foam plugs and kept in a BOD at 23 ± 2 °C, 60 ± 5% relative humidity (RH), and 12H scotophase. Mortality was evaluated after 48 h of exposure. Flies were considered dead if they did not show any movement even after being prodded with a fine brush.

2.4. Exposure of Old Mated Adults to Eucalyptus EO Low Concentrations

Once the dose–response curve of eucalyptus EO was determined, the concentrations LC_0 (control water + DMSO), LC_5, and LC_{20} were selected to assess the effect of low dose exposure on the survival and reproductive output of exposed flies. The exposure was performed as previously described, with the difference that females and males of the same age (5–7 days) were exposed separately to each concentration. After 48 h, mortality was evaluated and the survivors were transferred to new containers with sugar water solution (20% *w*/*v*). After 24 h, ten pairs (10 females and 10 males) from each concentration were formed and transferred to glass containers (200 mL) with 30 mL of artificial diet, with five repetitions for each concentration. The daily mortality of the parents was evaluated, and every 8 days the survivors were transferred to a new glass with diet present. For each concentration, 10 pupae were taken to estimate their mass. The daily number of emerged flies for each treatment was counted, and the bodyweights of newly emerged (less than 1 day) females (50) and males (50) were also determined.

2.5. Exposure of Newly Emerged Virgin Adults to Eucalyptus EO Low concentrations

Based on the result of the previous bioassay (see Section 3), the LC_{20} of eucalyptus EO was selected to test the effects of low concentration on biological and reproductive outputs of newly emerged virgin flies. Pupae (>1000) from *D. suzukii* rearing were individualized near emergence. Upon emergence, the newly emerged flies were sexed into four groups of 30 of either males or females, and were exposed to the LC_0 (control water + DMSO) or LC_{20} of the EO as previously described. After 48 h of exposure, the surviving adults were paired in the four following combinations: 1—untreated pairs (♀NT x ♂NT), considered as control and were exposed only to water + DMSO; 2—untreated females and treated males (♀NT x ♂T); 3—treated females and untreated males (♀T x ♂NT); and 4—treated males and females (♀T x ♂T). The different pairs were transferred to glass containers (10 pairs of the same combination per container of 200 mL) with diet present, and five repetitions per combination were made. After 8 days, the surviving adults of the same container were transferred to a new container with diet present, and this process was repeated four times. Parental adult longevity and fertility (weekly emergence) and the body mass of male (40) and female (40) adults from their progeny were determined.

2.6. Statistical Analysis

The results of the mortality bioassays were subjected to Probit analyses to the concentration–response curve and chi-square (χ^2) values with 95% confidence limits using the SAS V9 statistical software package (SAS Institute, Cary, NC, USA). The results of the survival were subjected to survival analysis using Kaplan–Meier estimators (Log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The total number of flies emerged and pupal and adult weights were subjected to univariate analysis of variance (ANOVA) or a Kruskal–Wallis one-way ANOVA on ranks, when the assumptions of normality and homoscedasticity were not satisfied. Pairwise comparisons were performed using Tukey's analysis of means ($p \leq 0.05$).

3. Results

3.1. Toxicity Bioassays

The mortality levels obtained in the concentration–mortality bioassay were satisfactorily described by the probit model with the goodness-of-fit test exhibiting a low χ^2-value ($\chi^2 = 1.10$) and a p-value ($p = 0.98$) higher than 0.05. Mortality in the control group did not exceed 10%. The tested *E. globulus* EO had a good lethal effect on adults of *D. suzukii* presenting an LC_{50} of 0.67μL·mL^{-1} and an LC_{90} of 1.57 μL·mL^{-1} (Table 1).

Table 1. Toxicity of essential oil of eucalyptus *Eucalyptus globulus* to adult flies of *Drosophila suzukii.*

Lethal Concentrations	No. of Insects	EO Concentrations (μL·mL^{-1})	Fiducial Interval (95%)	χ^2	p
	805			1.10	0.98
LC_5		0.22	0.17–0.26		
LC_{20}		0.38	0.32–0.43		
LC_{50}		0.67	0.61–0.71		
LC_{90}		1.57	1.40–1.83		

3.2. Effects of Low Concentrations of Eucalyptus EO on the Biological and Reproductive Traits of Old Mated Flies

3.2.1. Parental Flies' Longevity

The longevity of *D. suzukii* adult flies (5–7 days old) was not affected by exposure to low concentrations (LC_5 and LC_{20}) of *E. globulus* EO (Figure 1). No significant statistical differences were found for either the female (Log-Rank test, $\chi^2 = 1.93$; $df = 2$; $p = 0.39$; Figure 1A) or the male (Log-Rank test, $\chi^2 = 5.13$; $df = 2$; $p = 0.08$; Figure 1B) flies exposed to LC_5 and LC_{20} of eucalyptus EO when compared with the unexposed controls. Moreover, males generally exhibited lower longevity than females in all the treatments. Median survival time (LT_{50}) ranged from 15.20 to 16.43 days for females and from 9.94 to 11.53 days for males.

3.2.2. Parental Flies' Fertility

Females' fertility was assessed by counting the number of emerged females and males and the total (females + males) adults at 8 and 16 days as well as the aggregate of the numbers of adults emerged during the whole experiment (Day 8 + Day 16; Figure 2). Both at Days 8 and 16, no statistical significant differences were found for the total number of males and females (Day 8: $F = 2.262$; $df = 2$; $p = 0.147$; and Day 16: $F = 2.182$; $df = 2$; $p = 0.252$; Figure 2A), the number of females (Day 8: $F = 3.462$; $df = 2$; $p = 0.065$; and Day 16: $F = 0.825$; $df = 2$; $p = 0.462$; Figure 2B), and the number of males (Day 8: $F = 0.970$; $df = 2$; $p = 0.407$; and Day 16: $F = 3.327$; $df = 2$; $p = 0.071$; Figure 2C) of emerged flies between the treatments exposed to LC_5 and LC_{20} of essential oil and the unexposed control (LC_0). Interestingly, when the aggregate of the numbers of adults that emerged during the whole experiment (Day 8 + Day 16) were compared, significant statistical differences were found between exposure to the control (LC_0) and the LC_{20} for the total (females + males: $F = 8.844$; $df = 2$; $p = 0.004$; Figure 2A), for female ($F = 14.463$; $df = 1$; $p = 0.005$; Figure 2B), and for male ($F = 7.797$; $df = 1$; $p = 0.023$; Figure 2C) flies produced.

3.2.3. Progeny Pupal and Flies' Body Mass

The analysis of variance results showed that there is a significant decrease in pupae mass ($F = 7.83$; $df = 2$; $p = 0.009$) when parental *D. suzukii* adults were exposed to low concentrations (LC_5 and LC_{20}) of *eucalyptus* EO compared to unexposed flies (LC_0)

(Supplementary Figure S1). Such differences were not carried over to the female (F = 0.060; *df* = 2; *p* = 0.94) or male (F = 0.006; *df* = 2; *p* = 0.99) emerged flies (Supplementary Figure S1).

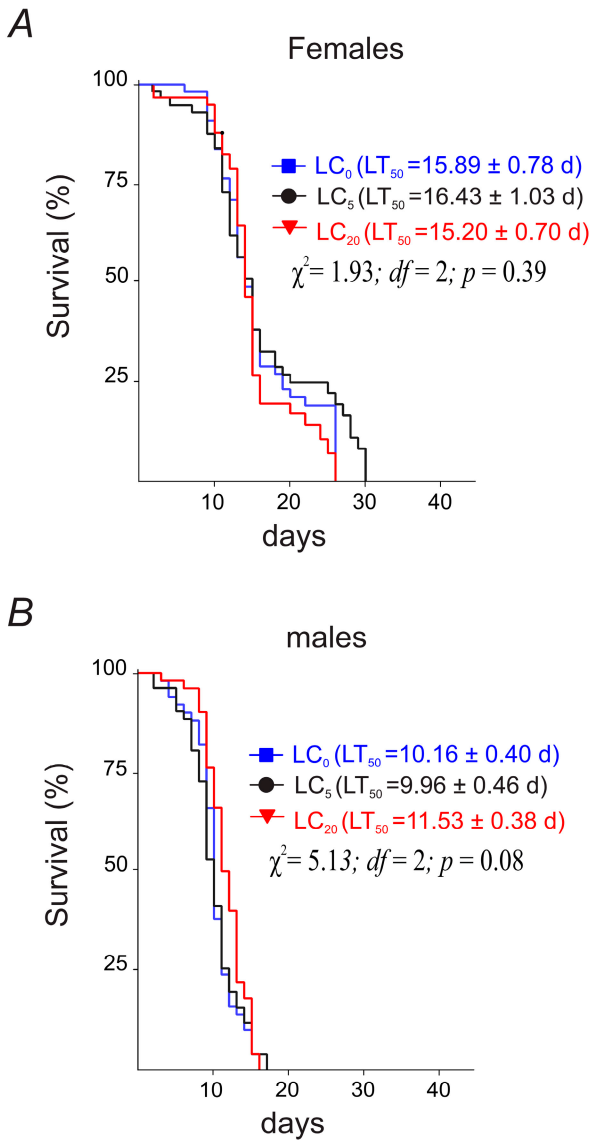

Figure 1. Longevity of 5–7-day old mated females (**A**) and males (**B**) of *Drosophila suzukii* exposed to low concentrations (LC_0, LC_5 and LC_{20}) of *Eucalyptus globulus* essential oil.

3.3. Effects of Low Concentration of Eucalyptus EO on the Biological and Reproductive Traits of Newly Emerged Virgin Adults

3.3.1. Parental Flies' Longevity

Significant differences were found between the four treatments for the longevity of the newly emerged and virgin female (Log-Rank test, χ^2 = 70.81; *df* = 3; *p* < 0.001; Figure 3A) and male (Log-Rank test, χ^2 = 21.40; *df* = 3; *p* < 0.001; Figure 3B) flies under different schemes of exposure to LC_{20} of the eucalyptus EO. Median survival time (LT_{50}) ranged from 37.71 to 21.70 days for females and from 15.52 to 24.62 days for males. Females from couples where only the female was exposed (♀T x ♂NT) lived significantly longer than the females from the control couples (♀NT x ♂NT) and other couples (♀NT x ♂T and ♀T x ♂T), while males of the control couples (♀NT x ♂NT) presented the highest longevity compared to the other combinations.

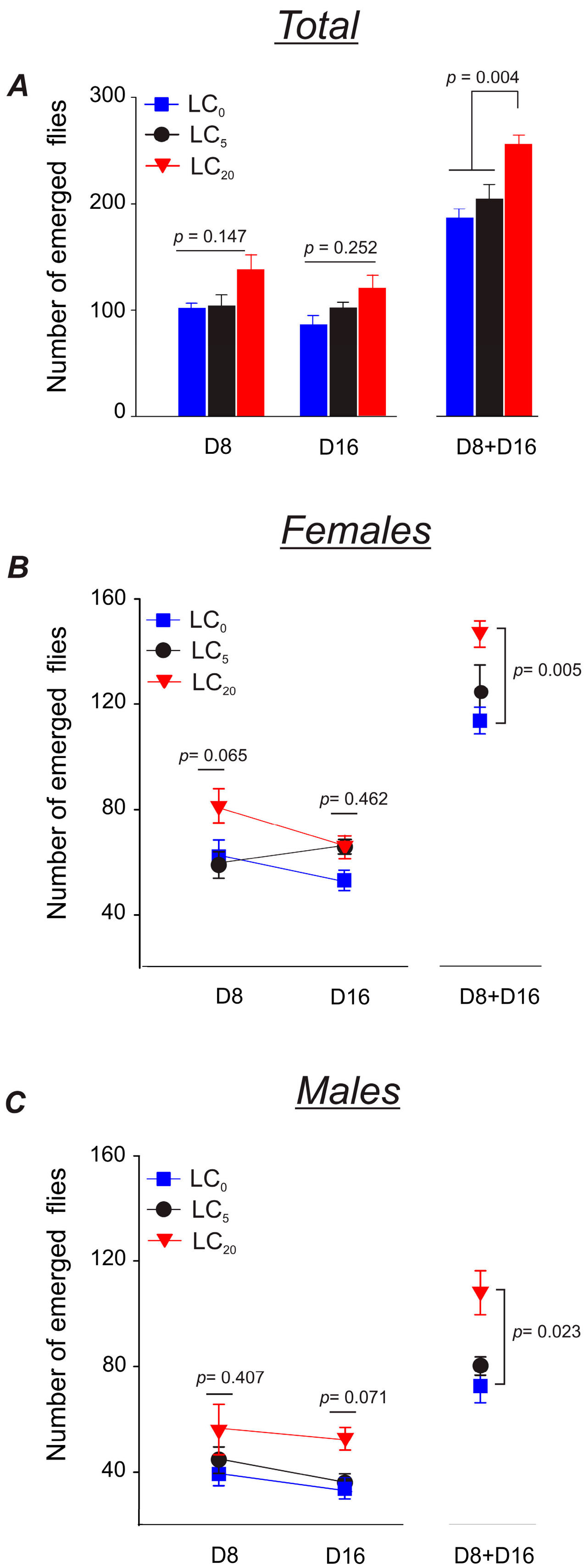

Figure 2. Total number (**A**), number of females (**B**), and number of males (**C**) of emerged progeny flies of 5–7-day old mated *Drosophila suzukii* exposed to low concentrations (LC_0, LC_5 and LC_{20}) of *Eucalyptus globulus* essential oil.

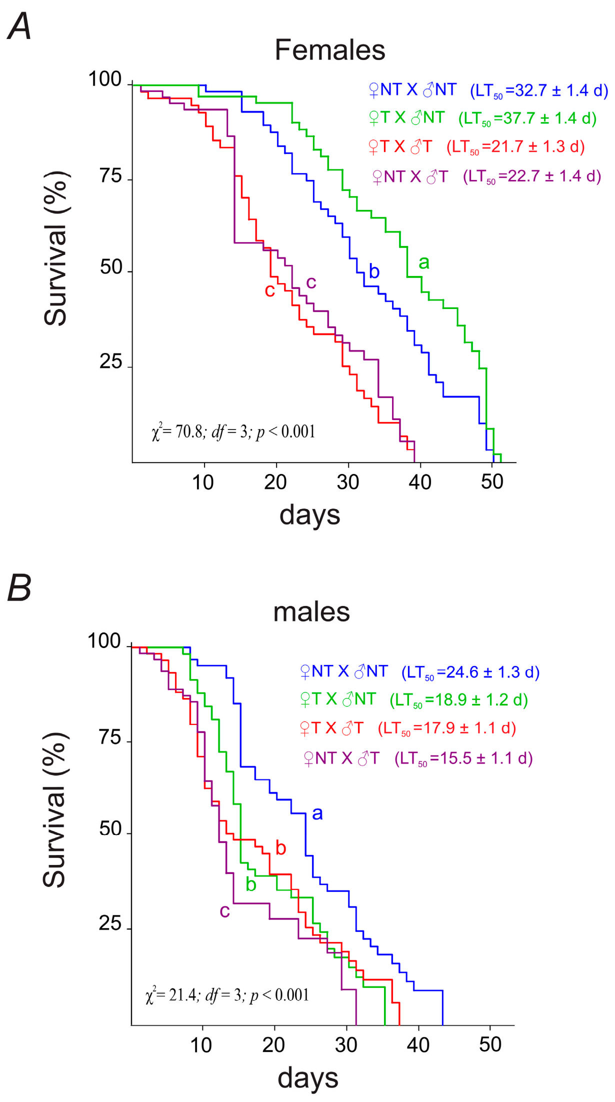

Figure 3. Longevity of newly emerged virgin *Drosophila suzukii* females (**A**) and males (**B**) paired in four couples' combinations: unexposed couples (♀NT x ♂NT), exposed females (♀T x ♂NT), exposed males (♀NT x ♂T), and exposed couples (♀T x ♂T) to the low concentration LC_{20} of *Eucalyptus globulus* essential oil.

3.3.2. Parental Flies' Fertility

The flies' emergence during the four weeks of the bioassay showed similar general trends between the four treatments for females and males as well as their combined total with some slight differences (Figure 4). In fact, the total number of flies emerged (females and males) did not differ between the four treatments in the two first weeks of emergence (Day 8: $F = 0.764$; $df = 3$; $p = 0.531$; and Day 16: $F = 2.678$; $df = 3$; $p = 0.082$) but presented contrasting differences in the third ($H = 8.758$; $df = 3$; $p = 0.033$) and fourth ($F = 7.054$; $df = 3$; $p = 0.003$) weeks (Figure 4A).

The untreated couples (♂NT x ♀NT) showed higher female ($F = 2.328$; $df = 3$; $p = 0.113$; Figure 4B) and male ($F = 2.453$; $df = 3$; $p = 0.101$; Figure 4C) emergences in the second week, resulting in a numerically, but not statistically, higher total number of emerged flies. Similarly, the couples where only males were exposed (♂NT x ♀T) produced more flies (Figure 4A) in the third week compared to the other combinations, and this higher number of emerged adults was mainly due to females' emergence ($F = 8.960$; $df = 3$; $p = 0.001$; Figure 4B). In the fourth week, the number of flies generated by couples where only males were exposed (♂NT x ♀T) and couples where both females and males were exposed

(♂T x ♀T) significantly decreased compared to the other two treatments due to a decrease in both produced females (F = 4.823; *df* = 3; *p* = 0.014; Figure 4B) and males (F = 9.430; *df* = 3; *p* < 0.001; Figure 4C).

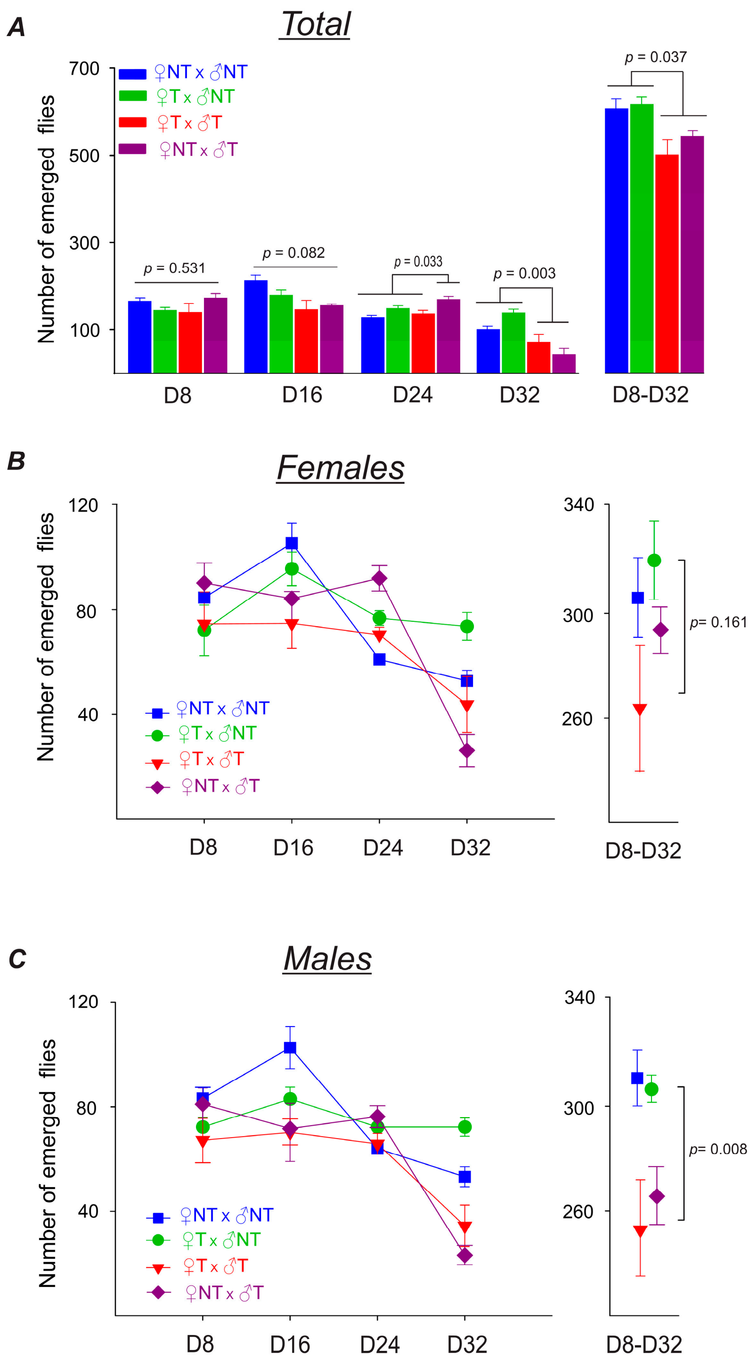

Figure 4. Total number (**A**), number of females (**B**), and number of males (**C**) of emerged progeny flies of newly emerged virgin *Drosophila suzukii* females (**A**) and males (**B**) paired in four couples' combinations: unexposed couples (♀NT x ♂NT), exposed females (♀T x ♂NT), exposed males (♀NT x ♂T), and exposed couples (♀T x ♂T) to the low concentration LC_{20} of *Eucalyptus globulus* essential oil.

When the aggregate of the numbers of adults emerged during the whole experiment (Days 8 to 32) were compared, significant statistical differences were found for the total (females + males: F = 3.581; *df* = 2; *p* = 0.037; Figure 4A) and for male (F = 10.787; *df* = 2; *p* = 0.008; Figure 4C) but not for female (F = 1.961; *df* = 2; *p* = 0.161; Figure 4B) flies produced.

The couples where only females were exposed (♂T x ♀NT) performed equal to the controls (♂NT x ♀NT) and produced the highest numbers of flies.

3.3.3. Progeny Flies' Body Mass

The body mass of the adult flies of the progeny did not differ between the four treatments for female (H = 2.59; df = 3; p = 0.47) or male (F = 1.99; df = 2; p = 0.58) emerged flies (Supplementary Figure S1).

4. Discussion

Plant-based products have been suggested as suitable alternatives for controlling insect pests worldwide because of their potential safety for the environment and human health. Although their antibacterial, antifungal, antiviral, antioxidant, and insecticidal properties have been frequently reported, their unintended effects are generally overlooked. The current investigation reports on the lethal and low concentrations effects of eucalyptus essential oils on a population of the spotted wing fly *D. suzukii*. Although our findings indicated good insecticidal toxicity of the eucalyptus EO against the fly's adults, exposure to low concentrations of this EO induced positive responses on the survival and reproductive output of exposed flies. Such beneficial responses depended on the age, sex, and mating status of the flies.

Our results demonstrated that the EO of eucalyptus was toxic for the exposed adult flies, and its low concentrations negatively affected the pupal descendants of exposed adults. Several natural compounds, such as essential oils, their major constituents, and nanoformulations, were previously reported as repellents, contact or ingestion toxicants, fumigants, ovicides, or oviposition deterrents for the spotted wing drosophila [17–23]. The tested plant species included members of the Myrtaceae family like eucalyptus plants. Eucalyptus oils and/or their major components have been reported to have insecticidal activity [24,30] including against *D. suzukii* [31,32]. As the activities of plants' extracts are strongly related to their chemical compositions, only the citral-based chemotypes of the eucalyptus EO were previously reported to have effective insecticidal activity [30,33]. However, in the present investigation, we tested a 1,8-cineole-based chemotype that showed good bioactivity against *D. suzukii* with an LC_{90} = 1.57 $\mu L \cdot mL^{-1}$. Such differences with the previous literature could be explained by the high percentage of the 1,8-cineole (94.4%) of the eucalyptus EO tested. Indeed, when used as a pure compound in contact bioassays, cineole was very toxic to spotted wing flies [31], and plants that presented high cineole content showed potent insecticidal [21,31] and repellent [34] activities against this insect.

Essential oils or their individual constituents are frequently advocated as cheap eco-friendly and low-risk replacement options for synthetic pesticides in the control of insect pests and disease vectors. Such perceived advantages derive primarily from the EOs' positive attributes linked with their natural origin. Thus, the EOs' bioactivities have been widely tested under laboratory conditions, and most studies focused on determining their lethal effects, and few tested the effects of sublethal exposure to essential oils. Our findings, from two different bioassays, report positive effects of exposure to low concentrations on the total number of produced flies and the longevity of exposed female adults. In ecotoxicological studies with synthetic insecticides, stimulatory responses at low doses have been reported in several insect pests [5–7]. Recently, a growing body of literature is indicating that similar responses can be induced by plant extracts including essential oils in insects [8–11,35–37] and other model organisms [38–40].

Exposure to low doses can favor different parameters including longevity, survival, reproduction, and population growth rates of exposed individuals [41,42]. In the present study, the beneficial responses induced by the low concentration of eucalyptus EO on *D. suzukii* resulted either in improved longevity or higher reproductive outputs. In previous studies on the plant extract-induced stimulatory responses, phytochemicals have been described to extend the lifespan also of *Anastrepha ludens* [43], *Drosophila melanogaster* [9,35,44], *Sitophilus zeamais* [8], and *Callosobruchus maculatus* [45] as well as to stimulate longevity, oviposition, and/or fecundity in *Ceratitis capitata* [11] and *Bactrocera oleae* [10].

Furthermore, the stimulatory effect of eucalyptus EO low concentration observed here depended on the age, sex, and mating status of the flies. Indeed, when 5–7-days old and mated *D. suzukii* adult flies were exposed to eucalyptus EO low concentration (LC_{20}), the total number of their progeny was higher compared to those of untreated flies while their longevity was not impacted. On the contrary, when virgin unmated flies were exposed to the low concentration of the EO, only longevity of exposed females was enhanced. Differential stimulatory or beneficial effects have been reported to occur in males and females after exposure to low levels of synthetic insecticides [46,47]. It is important to highlight that, in our study, the exposure to low concentration was based on the established concentration–response curve where 5 to 7-day old unsexed individuals were used. Such experimental conditions would explain, although partially, the sex- and age- dependent effects. Actually, *D. suzukii* response to chemical exposure has been shown to depend on the age and sex of used individuals [48] and that the interval between stress exposure and mating can affect parents' fitness in *C. maculatus* [45].

The age-, sex-, and mating status-specific effects might be also linked to a trade-off between life parameters of the flies leading to a prolonged life but at the cost of reproduction or vice-versa, a high reproductive output accompanied with a shorter life span. Trade-offs between different biological traits under stress circumstances have been recorded in insects [49–51] and were associated with either an increase of juvenile hormone levels [50,51] or a shift in resource allocation [51] when they occur between longevity and reproduction. The mechanisms underlying hormesis induced by phytochemicals are not well understood. Positive responses to low lethal concentrations, like prolonged life and/or higher fecundity, could result from exposed insect's induced immune, antioxidative, and other downstream adaptive responses. A generalized mechanism based on the redox-activated transcription factor Nrf2 (Nuclear factor erythroid 2-related factor2) was suggested by Calabrese and Kozumbo (2021) for hormesis in humans [52]. Nrf2 is a transcription factor that regulates the cellular defense against toxic and oxidative stress [53] and coordinates an evolutionarily conserved transcriptional activation pathway that mediates antioxidant and detoxification responses in many animal species, including insects [54]. However, such mechanisms still need to be further investigated and their validity proved in phytochemical induced stimulatory responses in insects.

5. Conclusions

In the present study, we report an age-, sex-, and mating status-related enhancement of longevity and fecundity in the spotted wing drosophila mediated by the exposure to low dose of eucalyptus EO. Our findings reinforce the idea that the potential unintended effects and risks associated with so called biorational compounds such as plants' EOs need to be thoroughly assessed before advocating them as alternatives to synthetic compounds. The knowledge about such beneficial responses in insect pests is critical to determine the implications of the implementation and sustainability of essential oils within insect pest management programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13020404/s1, Figure S1: Body mass of pupae (A), adults flies (B) progeny of *Drosophila suzukii* exposed to low concentrations (LC_0, LC_5 and LC_{20}) of *Eucalyptus globulus* essential oil and body mass adults progeny flies (C) of newly emerged virgin *D. suzukii* paired in four couples combinations: unexposed couples (♀NT x ♂NT), exposed females (♀T x ♂NT), exposed males (♀NT x ♂T), and exposed couples (♀T x ♂T) to the low concentration LC_{20} of *E. globulus* essential oil.

Author Contributions: M.P.: methodology, investigation, formal analysis, writing—original draft; E.L.d.A.A.: investigation, data curation; J.A.A.: investigation; V.d.C.C.: investigation, data curation; K.H.: resources, conceptualization, methodology, validation, supervision, formal analysis, writing—original draft. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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