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BIOLOGICAL RESPONSES OF *Drosophila suzukii* (MATSUMURA, 1931) (DIPTERA: DROSOPHILIDAE) TO STRESSES INDUCED BY SYNTHETIC AND BOTANICAL INSECTICIDES AND TEMPERATURE

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Prof. Dr. Khalid Haddi
Orientador

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Pineda, Arteaga Maria Rosario
Biological responses of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) to stresses induced by synthetic and botanical insecticides and temperature/ Maria Rosario Pineda Arteaga. - 2024.
177 p.

Orientador(a): Khalid Haddi.
Tese (doutorado) - Universidade Federal de Lavras, 2024.
Bibliografia.

1. Mosca-de-asas-manchadas 2. Toxicidade. 3. Hormese. I. Haddi, Khalid. II. Título.

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RESPOSTAS BIOLOGICAS DE *Drosophila suzukii* (MATSUMURA, 1931) (DIPTERA: DROSOPHILIDAE) SOB ESTRESSES INDUZIDOS POR INSETICIDAS SINTETICOS, BOTANICOS E TEMPERATURE

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APROVADA em 09 de janeiro de 2024.

Dr. Ricardo Siqueira da Silva (UFVJM)
Dr. Marcus Alvarenga Soares (UFVJM)
Dr. Oscar Amaya Santos (Universidad de Pamplona, Colombia)
Dr. Alexander Huamán Mera (Universidad Nacional de Jaén, Perú)

Orientador
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LAVRAS – MG
2024

A todas las personas que hemos superado las dificultades del conflicto armado en Colombia.

Vivimos en un país hermoso, y depende de nosotros superar las dificultades, aprender todos los días, ser mejores personas…y poder escribir un futuro diferente.

Porque Ningún niño debe ver morir a sus padres.

A todas las victimas que sobrevivimos al frente 48 de las FARC.

Mientras exista esperanza, ¡existirá vida!

Dedico

AGRADECIMIENTOS

Faltarian páginas para agradecer y citar a todas las personas que contribuyeron para llegar hasta aquí. Sin embargo, empezare por los más distantes,

Al mejor profe de Estádistica que conocí: Hernado Criollo †, gracias infinitas por creer en mí y darme ese empujoncito que necesitaba para salir de mi zona de confort.

A la Universidad de Nariño, Pasto-Colombia, ¡Mi segundo hogar! Siempre estaré orgullosa de mis orígenes sureños.

A Tito Bacca, orientador, amigo y maestro, quien siempre será mi ejemplo a seguir, por brindarme su apoyo incondicional con sus llamadas y consejos motivacionales.

A Mis amigas, que a pesar de mi ausencia siempre han estado disponibles para mí: Laura, Claudia y Vivi, gracias por estar siempre a pesar de la distancia. ¡Nos vemeremos en breve!

A Lore, Jaime, Antonia y el Juaquin, gracias por sentir saudades de mí, y darme motivos para un día volver.

A JJ y JJ, sin uds hubiera sido imposible sobre llevar la época de pandemia. Aprendí que en este caminar de la vida, no siempre la familia tiene vínculos sanguíneos. Basta, con coincidir, respetar y tener empatía por los demás. Nos veremos en el país de 5 estrellas; no pasen por alto que conocieron una pastusa “Buena Papa”, y sobre todo No olviden que “NO me gusta esperar…Pero igual te espero!!!

A todos esos Colombianos y Catrachos, con los que un día comparti comida, bebida, bailes, recocha y momentos felices en “La Casita”.

A Ariane, Bejna, Karolina, Jessica, Julia, Fernanda y nuestro grupo “Sextou” por todas las sextas de lujo que nunca olvidaré, y que pasamos al intentar olvidar la rutina y el estrés.

A Ana Paula, experta en Corel Draw, por su compañerismo, amistad, y todo ese apoyo impresindible en el laboratorio.

A Luciano y Leia, por su disposión, ustedes serán los mejores técnicos que conocí, aprendí mucho de su experiencia y de su vida. Ustedes son los angeles de la guarda en el DEN, gracias por facilitar la vida de nosotros los estudiantes de Pós.

A mis hijos adoptivos de Brasil: Thamiris, Chaylane, Julia, Vinicius, Emanuel y Elisabet, fueron parte importante no solo en la ejecución y planificación de experimentos, gracias por permitirme enseñarles un poco de la vida, un poco de español y nada de entomología, pero sobre todo GRACIAS por darse el tiempo de mejorar mi portuñol y enseñarme que Brasil es más que “Pos-graduación”.

Crianza: “No aceptes críticas de personas a las que no pedirías consejo, lucha por tus sueños, vive una vida feliz, siempre estaré para ti cuando me necesites”.

A mi asesor Dr. Khalid Haddi, por la paciencia infinita conmigo, y a toda la familia MEET.

A mis compañeras de doctorado Lara, Glace y Mariana, por la amistad y colaboración en estos cuatro años. ¡Sobrevivimos una “PANDEMIA”, seguro tenemos muchas anécdotas para contar!

A Isabel, nuestra secretaria, quien siempre estuvo pendiente de mi llegada, mi proceso de adaptación y sobre todas las cosas, su ayuda con todos los tramites burocráticos.

A todos los profesores del Programa de Pós-graduación en Entomologia DEN-UFLA, quienes contribuyeron en mi formación personal y académica.

A los miembros de las bancas de acompañamiento, y Jurados de evaluación del documento final, por todos sus comentarios y aportes para la culminación de esta etapa.

A Brasil, un país inmensamente diverso con las personas más gentiles que he conocido, gracias por darme una familia en los días felices e imposibles que la posgraduación exige.

A las entidades de financiamento por permitirme realizar mi doctorado en Brasil, y financiar mis desplazamientos en congresos y seminários: Consejo Nacional de Desarrollo Científico y Tecnológico –**CNPq-**; Coordinación para la Mejora del Personal de la Enseñanza Superior -**CAPES-**(Finance code 001) y La Fundación de Apoyo a la Investigación del Estado de Minas Gerais –**FAPEMIG** (APQ-02230-21).

Y a todas las personas que se cruzaron en mi caminar, todos sin duda fueron parte esencial de esta experiencia de vida.

Y cómo dijo García Márquez:

No debemos llorar porque ya se terminó, debemos sonreír por lo que sucedió.

Gracias infinitas a todos!!!

AGRADECIMENTOS

Seriam necessárias páginas para agradecer e citar todas as pessoas que contribuíram para que chegásse até aqui. No entanto, começarei com as mais distantes,

Ao melhor professor de estatística que já conheci: Hernado Criollo †, muito obrigado por acreditar em mim e me dar aquele empurrãozinho que eu precisava para sair da minha zona de conforto.

À Universidade de Nariño, Pasto-Colômbia, minha segunda casa! Sempre terei orgulho de minhas origens sureños.

A Tito Bacca, conselheiro, amigo e professor, que sempre será meu exemplo a ser seguido, por me dar apoio incondicional com suas ligações e conselhos motivadores.

Às minhas amigas, que, apesar da minha ausência, sempre estiveram ao meu lado: Laura, Claudia e Viviana, obrigado por estarem sempre presentes, apesar da distância. Até breve!

A Lore, Jaime, Antonia e Juaquin, obrigado por se sentirem bem comigo e por me darem motivos para voltar um dia.

Para JJ e JJ, sem vocês teria sido impossível superar a pandemia. Aprendi que, nesta jornada da vida, a família nem sempre tem laços de sangue. Basta concordar, respeitar e ter empatia pelos outros. Nos veremos no país das 5 estrelas; não se esqueçam de que conheceram uma Pastusa "Buena Papa" e, acima de tudo, não se esqueçam de que NO me gusta esperar…Pero igual te espero!!!

A todos os Colombianos e Catrachos, com quem um dia compartilhamos comidas, bebidas, danças, recocha e momentos felizes na "Casita".

À Ariane, Bejna, Karolina, Jessica, Julia, Fernanda e ao nosso grupo "Sextou" por todas as sextas de luxo que nunca esquecerei e que passamos tentando esquecer a rotina e o estresse.

À Ana Paula, especialista em Corel Draw, pelo companheirismo, amizade e todo o apoio indispensável no laboratório.

Ao Luciano e à Leia, pela disposição, vocês serão os melhores técnicos que conheci, aprendi muito com a experiência e a vida de vocês. Vocês são os anjos da guarda do DEN, obrigada por facilitarem a vida de nós, alunos da Pós.

Aos meus filhos adotivos do Brasil: Thamiris, Chaylane, Julia, Vinicius, Emanuel e Elisabet, vocês foram uma parte importante não só na execução e planejamento dos experimentos, obrigado por me permitirem ensinar um pouco da vida, um pouco de espanhol e nada de entomologia, mas acima de tudo OBRIGADA por dedicarem tempo para melhorar meu português e me ensinarem que o Brasil é mais do que "Pós-graduação".

Ao meu orientador, Dr. Khalid Haddi, por sua infinita paciência comigo, e a toda a família MEET.

Às minhas colegas de doutorado Lara, Glace e Mariana, por sua amizade e colaboração durante esses quatro anos. Sobrevivemos a uma "PANDEMIA", e tenho certeza de que temos muitas histórias para contar!

À Isabel, nossa secretária, que esteve sempre atenta à minha chegada, ao meu processo de adaptação e, principalmente, à sua ajuda em todos os trâmites burocráticos.

A todos os professores do Programa de Pós-Graduação em Entomologia DEN-UFLA, que contribuíram para a minha formação pessoal e acadêmica.

Aos membros dos júris de apoio e de avaliação do documento final, por todos os comentários e contribuições para a culminação dessa etapa.

Ao Brasil, um país imensamente diverso e com as pessoas mais gentis que já conheci, obrigada por me dar uma família nos dias felizes e impossíveis que a pós-graduação exige.

Aos órgãos de fomento por me permitirem fazer o doutorado no Brasil e por financiarem minhas viagens a congressos e seminários: Conselho Nacional de Desenvolvimento Científico e Tecnológico – **CNPq,** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – **CAPES** (Finance code 001) e a Fundação de Amparo à Pesquisa do Estado de Minas Gerais – **FAPEMIG** (APQ-02230-21).

E a todas as pessoas que cruzaram meu caminho e que, sem dúvida, foram parte essencial dessa experiência de vida.

E como disse García Márquez:

Não devemos chorar porque acabou, devemos sorrir pelo que aconteceu.

Agradecimentos infinitos a todos vocês!

RESUMO

A drosofila-de-asas-manchadas SWD *Drosophila suzukii* (Mastsumura, 1931) (Diptera: Drosophilidae), considerada uma praga polifaga de origem asiática, que tem preferência por atacar frutas vermelhas. Esta investigação foi realizada para avaliar compostos químicos alternativos ao Spinetoram, como os: organofosforados, piretroides, neonicotenoides, espinosinas, pirroles e diamidas, dois óleos essenciais: *Eucalyptus globulus; Citrus sinensis* (L.) e um composto majoritário (R)-(+)-limoneno para o controle de adultos de *D. suzukii*. Além disso, foram efetuados bioensaios para analizar alguns efeitos de concentrações subletais de espinosinas, óleos essenciais (OE) de eucalipto, laranja e o composto majoritário (R)-(+)-limoneno. Por último, determinamos o efeito da Temperature sobre o ciclo biológico e sobre as principais características morfológicas da espécie (asas, tórax e ovipositor). Os bioensaios foram realizados com uma criação de *D. suzukii* mantida no Laboratorio de Entomologia Molecular e Ecotoxicolgia (MEET), foram feitos. Moscas adultas (5-7 dias) foram expostas por ingestão e contato para determinar as curvas de dose-resposta de todos os compostos sob condições controladas (23-25ºC; 60-65% UR, 12h escotofase). Logo, adultos foram expostos para determinar os efeitos sub-letais das espinosinas (LC_5, LC_{10} e LC_{20}), do OE de eucalipto (LC_5, LC_{20}), do OE de laranaja e do seu composto majoritário (LC_1, LC_5, LC_{10} e LC_{20}). Posteriormente, avaliamos a longevidade dos adultos, a fecundidade das fêmeas e a massa corporal dos adultos recém-emergidos. Finalmente, duas populações de moscas adultas *D. suzukii* foram aclimatadas (20 e 25ºC), e o monitoramento foi realizado a cada 5 gerações, de F_{10} a F_{30}. Determinamos o ciclo de vida, a massa corporal e a longevidade dos adultos, e foram quantificados parâmetros morfométricos relacionados à plasticidade fenotípica: tamanho do corpo (medidas do tórax), comprimento, largura e área total da asa, área da mancha nas asas de machos, largura e número de dentes do ovipositor nas fêmeas. Nossos resultados indicam que todos os compostos testados têm apresentam toxicidade e têm potencial para o controle de adultos de *D. suzukii*, no entanto, em baixas concentrações as espinosinas e OE's producem um efeito estimulante na sobrevivência de adultos e na reprodução. Finalmente, confirmamos que a Temperature causa um efeito na plasticidade fenotipica desta espécie, levando a alterações na morfologia, dando origem a morfotipos que podem ser facilmente adaptáveis as condições ambientais e garantindo o sucesso reprodutivo desta espécie.

Palavras-chave: Mosca-de-asa-manchada. Toxicologia. Hormese, Óleos essenciais. Morfometria.

GENERAL ABSTRACT

The spotted wing drosophila (SWD) *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), considered a polyphagous pest of Asian origin, has a preference for attacking red fruits. This research was carried out to identify alternative chemical compounds to spinetoram, such as organophosphates, pyrethroids, neonicotinoids, spinosyns, pyrroles and diamides, two essential oils: *Eucalyptus globulus*; *Citrus sinensis* (L.) and a major compound (R)-(+)-limonene for the control of *D. suzukii* adults. In addition, bioassays were carried out to determine the effect of sublethal concentrations of spinosyns, essential oils (EO) of *Eucalyptus*, orange, and the major compound (R)-(+)-limonene. Finally, we determined the effect of temperature on the biological cycle and the main morphological characteristics of the species (wings, thorax, and ovipositor). The bioassays were carried out using stock from the Molecular Entomology and Ecotoxicology Laboratory (MEET). Adult flies (5-7 days old) were exposed by ingestion and contact with the compounds to determine the dose-response curves of all the compounds under controlled conditions (23-25°C; 60-65% RH, 12h scotophase). Adults were then exposed to determine the sub-lethal effects of spinosyns (LC_5, LC_{10}, and LC_{20}), *Eucalyptus* EO (LC_5, LC_{20}), orange EO and its majority compound (LC_1, LC_5, LC_{10}, and LC_{20}). Subsequently, we evaluated the longevity of the adults, the fecundity of the females, and the body mass of the newly-emerged adults. Finally, two populations of adult *D. suzukii* flies were acclimatized (20 and 25°C), and monitoring was carried out every 5 generations, from F_{10} to F_{30}. We determined the life cycle, body mass, and longevity of the adults, and quantified morphometric parameters related to phenotypic plasticity: body size (thorax measurements), width, area length, wing shape, and size, wing spot area in males, width and number of ovipositor teeth in females. Our results indicate that all the compounds tested are toxic and can potentially control *D. suzukii* adults; however, at low concentrations, spinosyn and EOs produce a stimulating effect on adult survival and reproduction. Finally, we confirmed that temperature has an effect on the phenotypic plasticity of this species, leading to changes in morphology, giving rise to morphotypes that can be easily adapted to environmental conditions, and guaranteeing the reproductive success of this species.

Keywords: Spotted wing drosophila. Toxicology. Hormesis. Essential oil. Morphometry.

SUMÁRIO

FIRST PART

GENERAL INTRODUCTION

Drosophila suzukii (Matsuma, 1931) (Diptera: Drosophilidae), known as the spotted wing *Drosophila* (SWD), is an exotic pest of Asian origin (Japan). It was first described attacking cherry berries in 1916. However, in recent years it has spread around the world. In 2008, several invasions were recorded in Europe and the United States, and from 2013 it was detected in South America, with the first captures in Brazil being made in the states of Rio Grande do Sul and Santa Catarina, and in Uruguay (HAUSER, 2011; CALABRIA G, MACA J, BACHLI G, 2012; DEPRÁ et al., 2014; SCHLESENER, D. C. H. WOLLMANN et al., 2015; ANDREAZZA et al., 2016). The pest is currently reported in Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Goiás, and Minas Gerais, regions where various native and exotic fruit crops can provide conditions for the rearing and establishment of *D. suzukii*, a fact that raises concern (NAVA, DORI EDSON; BOTTON, M., BERNARDI, D., ANDREAZZA, F. & BARONIO, 2015; SCHLESENER et al., 2018). It is considered a difficult pest to manage because, unlike other species of the *Drosophila* genus, which require ripe and decaying fruit to oviposit, it uses healthy fruit as a substrate for oviposition. The female, with its serrated and sclerotized ovipositor, pierces the exocarp of the healthy fruit to lay the eggs. This is followed by the larvae, which consume the inside of the fruit and cause the most damage. Thus, the attack of this pest depreciates the quality of the fruit, reducing the useful life of the product in the post-harvest period (GERDEMAN, B. S.; TANIGOSHI, 2010; ANDREAZZA et al., 2017b; PÉREZ et al., 2019).

Thus, this fly is considered a polyphagous pest that attacks a variety of native and commercial fruit species. However, it prefers red fruits (FAHRENTRAPP, J. et al., 2019). It has a short life cycle (10 to 12 days; 25^{o}C) and high reproductive potential (each female oviposits an average of 635 eggs), which gives it the ability to invade and spread rapidly into new regions (EMILJANOWICZ et al., 2014), and makes it potentially dangerous for Brazil's fruit-growing sector, taking into account that Brazil is a country of continental proportions, and ranks third in the world for fruit production. Production is diversified due to the country's climatic conditions, which make it possible to produce tropical, subtropical, and temperate fruit, which represents favorable conditions and a variety of substrates for the rearing and invasion of *D. suzukii*. Regarding the management of this pest, the most efficient control strategy is the use of synthetic insecticides from different chemical groups (SCHLESENER et al., 2019). However, other strategies that have been successful for its control beingtimely harvesting, pruning, irrigation, mulching and exclusion netting, removal of ripe, overripe, or fermented fruit that can be the host for SWD reproduction, and mass trapping (SCHÖNEBERG et al., 2020; TAIT et al., 2021), control of weeds and alternative hosts in orchards in addition to monitoring aimed at sampling fruit with the presence of larvae, and in the case of adults with plastic traps using attractants such as apple or grape vinegar (TAIT et al., 2018).

Biological control is also an important part of the strategies for SWD control, and so far the most important organisms are parasitoid wasps such as *Leptopilina boulardi* (Costa Lima) (Hymenoptera: Figitidae); *Trichopria anastrephae* (Perkins) (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae Rondani* (Hymenoptera: Pteromalidae) (MARCHIORI; BARBARESCO, 2007) (LEE et al., 2019). Commercially available predators such as *Orius insidiosus* (Say), *Orius majusculus* (Reuter), *Orius laevigatus* (Fiber) and *Anthocoris nemoralis* (Fabricius, 1794) (Hemiptera: Anthocoridae), *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) have been reported experimentally to control SWD (GABARRA et al., 2015; RENKEMA; CUTHBERTSON, 2018; ENGLERT; HERZ, 2019). In addition, the application of entomopathogenic fungi, such as *Beauveria bassiana* has shown some positive results in this fly control (MARCHIORI; BARBARESCO, 2007; COSSENTINE; ROBERTSON; BUITENHUIS, 2016; BERNARDI et al., 2017). In recent years, the use of plant-based products as new alternatives for the control of *D. suzukii* has been extensively investigated. Thus, more than 20 essential oils have been evaluated for their repellent, toxic, fumigant, ovicidal, and oviposition-impeding properties (MADEMTZOGLOU et al., 2013; RENKEMA et al., 2016; DAM; MOLITOR; BEYER, 2019; CAETANO et al., 2022; DE SOUZA et al., 2022).

However, as with insecticides, it has been reported that exposure to low concentrations of these compounds can cause stimulatory responses. Positive effects on the reproduction and longevity of old and newly emerged adults of *D. suzukii* exposed to low concentrations of eucalyptus essential oil have already been reported (PINEDA et al., 2023). Therefore, despite the multiple studies that exist on the effect of hormesis on insects, questions remain due to the wide range of chemical products, ecological relationships, and environmental factors involved (CUTLER, 2013; GUEDES; RIX; CUTLER, 2022).

Considering that in Brazil, only spinetoram is registered for the control of *D. suzukii*, it is very important to identify which other insecticides and compounds may be efficient for its control. It is also necessary to consider the specific mechanisms and the effects that low concentrations in the long term can have on these new products.

Temperature is considered the most important environmental factor in insect development, and studies show that it can be directly related to hormesis (SILVA et al., 2024) or cause other visual effects in insects, specifically morphological changes (BUJARRABAL; SCHUMACHER, 2016). Thus, within phenotypic plasticity, allometry makes it possible to study changes in the size and shape of specialized insect structures. This research can be done to determine the effect of exposure to different compounds, or to external factors such as environmental stress (SIOMAVA; WIMMER; POSNIEN, 2016). One of the most widely used tools to assess these changes in the phenotype of a species is the use of morphometrics. In *D. suzukii*, several studies indicate that temperature has a strong relationship with and direct effects on the shape of the wings and ovipositor, and on the life cycle, generating morphotypes that may adapt easily to different altitudinal gradients (CLEMENTE et al., 2018; FRAIMOUT et al., 2018; COSTANTINI, 2019).

In this way, this research thesis presents four scientific articles as the results of various bioassays specifically focused on three areas: 1. Toxicology of insecticides and essential oils; 2. Sub-lethal effects of spinosyns and essential oils and, 3. Effect of temperature on the morphology of *D. suzukii* adults.

1. THEORETICAL FRAMEWORK

1.1 *Drosophila suzukii:* Origin, biology and distribution

D. suzukii, known as the spotted wing drosophila (SWD), is an exotic pest that was first described attacking cherry trees in 1916 in Japan (KANZAWA, 1934, 1935). However, in recent years, it has spread around the world. In 2008, there were first invasions to Europe and the United States, and from 2013 it was detected in South America, with the first captures in Brazil being made in the states of Rio Grande do Sul and Santa Catarina, and in Uruguai (HAUSER, 2011; CALABRIA G, MACA J, BACHLI G, 2012; DEPRÁ et al., 2014; SCHLESENER, D. C. H. WOLLMANN et al., 2015).

Brazil is a country of continental proportions and ranks third in the world for fruit production. Production is diversified due to the country's climatic conditions, which make it possible to produce tropical, subtropical, and temperate fruit, which represents favorable conditions and a variety of substrates for the breeding and invasion of *D. suzukii*. Currently, this pest has been reported in Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Goiás, and Minas Gerais, regions where a variety of fruit crops, both native and exotic, can provide conditions for the breeding and establishment of *D. suzukii*, a fact which raises concern because this pest, despite having a preference for red fruit, is considered a polyphage (DEPRÁ et al., 2014; NAVA, DORI EDSON; BOTTON, M., BERNARDI, D., ANDREAZZA, F. & BARONIO, 2015; SCHLESENER et al., 2018).

The adults of *D. suzukii* are characterized by having a soft body with red eyes, a light brown thorax, and a light brown abdomen with black transverse stripes (Figure 1). Approximately 2-3 mm long, this species is sexually dimorphic, the females are slightly larger than the males and are easily characterized by the presence of spot wing tips while females present two short sexual combs on the 1st and 2nd segments (respectively) of the anterior tarsus. Females are recognizable by their characteristic large, pointed, sclerotized, and serrated ovipositor, which allows them to break through the skin of healthy fruit. This species has a short life cycle (10 to 12 days; 25°C) and high reproductive potential (each female oviposits an average of 635 eggs) (Figure 1), which gives it the ability to be a potential pest (EMILJANOWICZ et al., 2014; WINKLER et al., 2021).

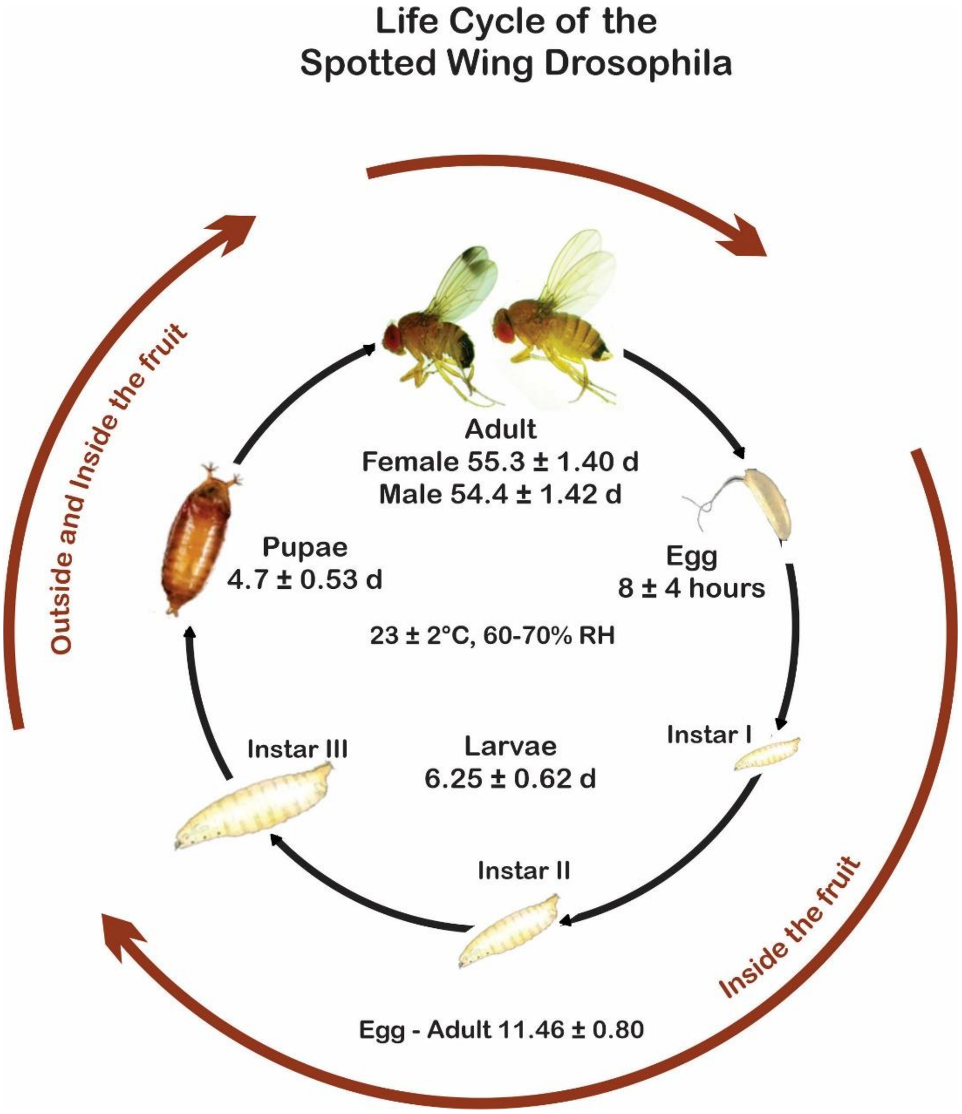

Figure 1. The life cycle of *Drosophila suzukii* in controlled conditions of 23±2ºC; 60±70% RH, and 12H scotophase (laboratory of Molecular Entomology and EcoToxicology (MEET). Federal University of Lavras)

1.2 Chemical control of *Drosophila suzukii*

Worldwide, the most efficient control for SWD is the use of broad-spectrum, targeted adult synthetic insecticides (SHAWER, 2020). They are generally applied in sprays with calendar applications. Most insecticides act on the nervous system, such as organophosphates, pyrethroids, and carbamates (ANDREAZZA et al., 2017). However, the most efficient ones are within the group of spinosyins: spinosad and spinetoram. Spinosad is derived as a product of the fermentation of the bacterium *Saccharopolyspora spinosa* Mertz & Yao and consists of spinosyns A and D. Spinoteram is a synthetic spinosyn consisting of spinosyns J and L. The latter exhibits greater insecticidal activity, acts more quickly, and has a longer duration of action compared to spinosad. However, both control a wide range of pests in fruits and vegetables and are used in organically certified crops (MERTZ; YAO, 1990; DRIPPS et al., 2008; BACCI et al., 2016; VAN TIMMEREN; ISAACS, 2013, GEISLER et al., 2015; WISE et al., 2015; HAYE et al., 2016; FANNING; GRIESHOP; ISAACS, 2018; SHAW et al., 2019; SIAL et al., 2019). More recent studies reported that growth regulators such as lufenuron, cyromazine, and pyriproxyfen showed efficiency in larval control while products such as azadirachtin and tebufenozide decreased offspring production (SÁNCHEZ-RAMOS; FERNÁNDEZ; GONZÁLEZ-NÚÑEZ, 2023)

In Brazil, according to Agrofit, only Spinetoram is allowed for the control of *D. suzukii* adults (AGROFIT, 2023). The spinosyns act at the binding sites of nicotinic acetylcholine receptors (nAChR), serving as allosteric modulators of nicotinic acetylcholine receptors. This results in a continuous and uncontrolled transmission of nerve impulses, inducing hyperexcitation of the insect's nervous system. This leads to fatigue and muscular paralysis, ultimately culminating in the insect's death (PERRY; MCKENZIE; BATTERHAM, 2007; YEE; ALSTON, 2009; WATSON et al., 2010; ANDREAZZA et al., 2017a). Repetitive use of a chemical molecule over time can result in the selection of resistant insects, which can make insecticides less efficient for pest control (KHAN et al., 2020; PANDIAN; RAMESH, 2020). In the last decade, spinosyns have been widely used to control various pests in major crops, causing the emergence of populations resistant to this class of bioinsecticides. For example, in Brazil, resistance to spinosyns (RR>50) has already been reported in field populations of the tomato moth *Tuta absoluta* since 2015 (REYES et al., 2012; SILVA et al., 2016). In addition, the first reports of *D. suzukii* resistance to spinosad in raspberry crops were in Watsonville (USA), where flies collected from spinosad-treated regions exhibited LC_{50} values 4.3 to 7.7 times higher than those from untreated regions and 11.6 to 22.4 times higher than the susceptibility reference values (GRESS; ZALOM, 2019, 2022; DISI et al., 2020). Taking into account that in Brazil Spinetoram is the only active ingredient registered for the control of *D. suzukii*, the development of resistance in Brazilian populations of *D. suzukii* becomes a real concern.

1.3. Derivates of natural products: Innovative control tools

In the last decades, agricultural systems have demanded the search for new pest control strategies to maintain crop production to feed the world. Traditional agriculture commonly uses synthetic insecticides because of their efficiency in controlling agricultural pests, however, some factors can affect their efficacy, such as over- or lower-dosing of application rates, incorrect formulation, and timing of application. All these problems, together with environmental factors such as climate change, have caused pests to develop tolerance and, in many cases, resistance to many insecticidal compounds (HUA; JONES; RELYEA, 2014; DAVID, 2021; GHALBANE et al., 2022). In this way, new alternatives for pest control have been sought, with a special focus on the use of natural products that allow an eco-friendly agricultural production. In this sense, much importance has been given to plants and their by-products. Essential oils (EOs) have been used since ancient times for their multiple bioactive properties. The use of essential oils has been reported in the pharmaceutical, food, culinary, and cosmetic industries. In the agricultural sector, many plants have been noted for their insecticidal properties (ISMAN, 2020a; KIRUTHIKA; VISHALI, 2023). Throughout history, we know that the most commonly used botanical insecticides are pyrethrum, extracted from dried flowers of *Tanacetum cinerariifolium* (Asteraceae) and neem (azadirachtin) from *Azadirachta indica*, (Meliaceae), (TOMINAGA, 1982; KILANI-MORAKCHI; MORAKCHI-GOUDJIL; SIFI, 2021). Only in the 1980s did essential oils emerge as a new alternative for pest control, however, there are many limitations to their direct use in the field (ISMAN, 2020b). It has been reported that they have insecticidal properties, but no research that specifies their mechanism of action, so they are only known to be multisite (ZENI et al., 2021; RHOUMA et al., 2023). The first challenge faced is to find the right dosage for each insect pest, followed by the type of application. Essential oils are unstable molecules and have low solubility in water. Large quantities of raw materials are required to obtain them, and higher and continuous production of plant biomass is necessary to guarantee constant production. Then, there is the fact of evaluating whether the doses found will not cause phytotoxicity, and finally, the formulation and commercialization process, which will depend largely on the technical norms of each country (LIBS; SALIM, 2017; ISMAN, 2020a). Among the most commonly used plants with biopesticidal effects are the Asteraceae, Myrtaceae, Apiaceae, Lamiaceae, and Rutaceae, composed mainly of monoterpenes, which are the molecules that affect pests by acting as repellents, fumigants, larvicides and adulticides (TRIPATHI, A.K. et al., 2009; MOSSA, 2016). Plant products of these families are frequently reported to have the potential to control different arthropod species such as mites, Coleoptera, Lepidoptera, Hemiptera, and Diptera (MOHARRAMIPOUR; NEGAHBAN, 2014; ISMAN, 2020b). In recent years, the efficiency of essential oils like *Eucalyptus globulus* and *Citrus* sp was investigated for the control of *D. suzukii* (BOŠKOVIĆ et al., 2023). Despite the advantages of being plant-derived and environmentally friendly, few studies have been conducted to determine their selective effect against non-target insects, nor the causes of sublethal effects (HADDI et al.,2020).

1.4. Sub-lethal effects of bioinsecticides

So far, there is no global term for bioinsecticides and different definitions and classifications are used depending on governmental entities, companies producing biological inputs, and regulatory agencies in each country (HADDI et al., 2020). However, all definitions agree that a bioinsecticide is composed of natural products or their derivatives, such as plants or microorganisms. They are divided into three groups: botanical, microbiological, and biochemical (PAMELA, 2019; CHOPRA; DHINGRA, 2021). Their components vary widely, as do their mechanisms of action (JOHNSON, 2021). Plant derivatives such as essential oils are composed of secondary metabolites (phenols, terpenes, and nitrogenous compounds), which act mainly as a defense mechanism against herbivorous insects (HOFSTETTER; FASSAUER; LINK, 2018). Thus, essential oils with insecticidal properties are characterized by a characteristic odor and are generally composed of monoterpenes (KHURSHEED et al., 2022). On the other hand, derivatives of microorganisms such as spinosyns, which are derived from soil bacteria (*Saccharopolyspora spinosa*), are also recognized as a less harmful ecological alternative to common insecticides and are efficient for the control of different types of pests, mainly fruit flies (MERTZ; YAO, 1990; CAGÁŇ et al., 2022). In consequence, biopesticides, despite causing lethal effects on unwanted pests and presenting multiple benefits, are biodegradable, eco-friendly, and safe for other organisms, and few studies report side effects when target and non-target insects are exposed to low concentrations. However, sublethal effects produce multiple responses (positive and negative) that alter insect development, reproduction, and behavior. The positive responses are called hormesis effects (RIX; GUEDES; CHRISTOPHER CUTLER, 2022). Thus, hormesis is a phenomenon that occurs as a biphasic response to stress, defined by stimulation at low doses and inhibition at high doses and is frequently observed in insects. In physiological terms, hormesis is a result of direct stimulation of cellular processes or defenses or, much more commonly, by an overcompensation of cellular processes or defenses following mild exposure to a stressor (CALABRESE, 2008) including exposure to low concentrations of essential oils and spinosyns. The implications of this phenomenon in the agricultural area are multiple, (GUEDES; RIX; CUTLER, 2022; HADDI et al., 2020). The hormetic responses range from changes in developmental time, to increased fecundity, fertility, longevity, and behavior (RIX; GUEDES; CHRISTOPHER CUTLER, 2022). Although, many studies reported hormetic effects caused by conventional insecticides (CALABRESE, 2008; BREVIK et al., 2018; GIBERT; DEBAT; GHALAMBOR, 2019), only a few studies reported these effects for biopesticides, and even fewer for essential oils (SILVA et al., 2017b, 2017a; DE SOUZA et al., 2022; PINEDA et al., 2023). Low concentrations of EOs (LC_{30} and LC_{20}) from the genera *Cupressus*, *Juniperus*, *Picea* and *Pinus* had a significant impact on the longevity, development and vitality of *Spodoptera littoralis* (Lepidoptera: Noctuidae), *Culex quinquefasciatus* (Diptera: Culicidae) larvae, and *Musca domestica* (Diptera: Muscidae) adults (PAVELA et al., 2021). Several essential oils presented stimuli in the longevity, fecundity, and fertility of different insect or mite species such as *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) (IZAKMEHRI et al., 2013), *Sitophilus zeamais* (Coleoptera: Curculionidae) (SILVA et al., 2017b), mites (Acari) (SHIRVANI et al., 2023), *Aphis fabae* (Hemiptera: Aphididae) (KHANI; ASGHARI, 2012; SWARTZ et al., 2019), *Spodptera exigua* (Lepidoptera: Noctuidae) (ROSAS-GARCÍA; TORRES-ORTEGA; VILLEGAS-MENDOZA, 2021), *D. suzukii* (SOUZA et al., 2022). In pollinators such as honeybees and bumblebees, adverse effects such as changes in foraging, neurological or cognitive effects, and hive communication failures, associated with sublethal exposure to various compounds have been reported (LU; HUNG; CHENG, 2020; GIUNTI et al., 2022). In addition to identifying compounds for the control of exotic pests such as *D. suzukii*, it is important to know the effects of residues left in the environment, to determine the effects on the pest and on the other arthropods that make up the trophic chain. This is essential to improve current IPM strategies.

1.5. Phenotypic plasticity in relation to temperature

The evolutionary capacity of insects has led them to evolve with the environment. Thus, phenotypic plasticity refers to the ability of an insect to exhibit different phenotypes in response to changes in its environment. It is determined by genes that directly govern the morphology of structures, and environmental factors such as temperature, photoperiod, and food quality. This characteristic is essential in insects biology and reproduction, considering that they can alter their biological, behavioral, reproductive, and morphological traits (GIBERT, 2020; MOCZEK, 2010; GÖRÜR, 2000). Insects are poikilotherms and cannot regulate their body temperature, which means that they depend on external temperature for all the biological processes. If there are temperature changes, their development, survival, reproduction, and growth will be directly affected.

One of the most commonly used techniques to determine whether phenotypic plasticity is influenced by environmental factors such as temperature is the use of geometric morphometrics "GM" (LAWING; POLLY, 2010; TATSUTA; TAKAHASHI; SAKAMAKI, 2018). The GM is based on measuring and analyzing the shape, position, size, and variability of different body structures (ROHLF; SLICE, 1990). In addition to linear measurements, the relative position of anatomical points is also used, which are derived from cartesian geometric coordinates of morphological structures instead of linear, areal or volumetric variables, taking into account landmarks, curves and contours in two (2D) or three (3D) dimensions (KLINGENBERG, 2011; ADAMS; OTÁROLA-CASTILLO, 2013; VILLALOBOS-LEIVA; BENÍTEZ, 2020). Once the photographs are digitized, with R or Morpho J software, a generalized overlay of points (Procrustes) is performed, which yields a principal component analysis (PCA), and multivariate analysis, which allows us to identify patterns of variation in shape and to study their covariation with environmental, genetic or developmental factors (ADAMS; OTÁROLA-CASTILLO, 2013; BENÍTEZ; PÜSCHEL, 2014; ZÚÑIGA-REINOSO; BENÍTEZ, 2015).

Thus, it has been proven that changes in temperature cause variations in insects' vital traits, such as incubation periods, instar duration, and total development periods from egg to adult. In morphological terms, changes can occur in the insect's body aspects such as shape, color, and size mainly of wings and abdomen (MALLARD; NOLTE; SCHLÖTTERER, 2020).

In pest management, knowing the morphotypes of pest species is very important, as it could indicate the environmental conditions and limitations along altitude and altitudinal gradients, allowing us to establish the distribution range of pest insects (BUCKLEY, 2022; AMIRI; KHEBIZA; MESSOULI, 2023). In general, most research shows that there is an inverse relationship between temperature and phenotypic plasticity parameters. All species have an optimum temperature for their development. However, if exposed to low temperatures, insects are altitudinally more limited, and their life cycle may be extended, due to the lack of energy sources to fulfill their metabolic activities. On the contrary, when the temperature increases, they present a greater altitudinal distribution, and their life cycle is reduced (BONATO; CHADOEUF, 2018; MALLARD; NOLTE; SCHLÖTTERER, 2020). On the other hand, exotic pests, such as *D. suzukii*, are easily established and distributed because of their adaptive capacity to various environmental conditions (LITTLE; CHAPMAN; HILLIER, 2020). In terms of phenotypic plasticity, the main organs that undergo visible morphological modifications are wings and spots in males, and ovipositor in females (FRAIMOUT et al., 2018; TRAN; HUTCHISON; ASPLEN, 2020; VARÓN-GONZÁLEZ et al., 2020).

Thus, in addition to seeking new control tools, it is necessary to understand the effect of temperature on the life cycle, to predict its behavior and dynamics and determine the best management strategy.

FINAL CONSIDERATIONS

This research focused mainly on the toxicology of synthetic insecticides and essential oils as alternative compounds for the control of *D. suzukii*. The results indicate that all tested compounds are efficient for the control of spotted-wing drosophila adults. This allows the inclusion of molecules other than Spinetoram in chemical control programs, to avoid the selection of populations resistant to this compound. However, current IPM plans should be restructured and lethal concentrations per region and crop should be defined, taking into account that we found alterations in aspects of the biology and reproduction of this fly when exposed to sublethal concentrations, also affecting non-target insects.

As a new control tool, essential oils are an excellent choice. EOs are derived from natural products, being more environmentally friendly and contributing to clean fruit production. The results indicate that both EOs and their major compounds affect the control of adults. However, like synthetic insecticides, they can cause stimulatory responses (hormesis) at low concentrations. In this sense, our contribution was to generate knowledge in the area of toxicology to determine lethal concentrations and to evaluate the effect in sublethal concentrations. Several challenges remain to be solved, among them: **1.** To perform bioassays on pupae, as a strategy to control pupae, for terrestrial applications. **2.** Develop formulation methodologies that allow the use of these products in the field. **3.** Also to carry out phytotoxicity and toxicology tests for non-target insects.

In relation to biological aspects, temperature plays an essential role in the development of this species. Thus, this research proved that if there are alterations outside the normal range (23-25°C) flies adapt easily to survive, but at an adaptive cost. The stress caused by temperature changes generates morphological changes, mainly in wings, or biological changes, alteration in the timing of each stage of the life cycle. It is important to know these changes to improve control strategies. To complement this research, it is recommended to perform behavioral evaluations to relate them to the morphological changes of the species. On the other hand, toxicity bioassays at different temperatures should be carried out to know the lethal concentrations, since the efficiency of synthetic insecticides is related to the lethal concentration of the insecticides.

REFERENCES

ADAMS, D. C.; OTÁROLA-CASTILLO, E. geomorph: an R package for the collection and analysis of geometric morphometric shape data. **Methods in Ecology and Evolution**, v. 4, n. 4, p. 393–399, 2 abr. 2013. doi: 10.1111/2041-210X.12035.

AGROFIT. **AGROFIT**.

AMIRI, N.; KHEBIZA, M. Y.; MESSOULI, M. The Impact of Climate Change on Insect Pests Damaging Crops. In: [s.l: s.n.] p. 73–101. 2023.

ANDREAZZA, F.; BERNARDI, D.; BARONIO, C. A.; PASINATO, J.; NAVA, D. E.; BOTTON, M. Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae). **Pest Management Science**, v. 73, n. 1, p. 146–152, jan. 2017a. doi: 10.1002/ps.4348.

ANDREAZZA, F.; BERNARDI, D.; DOS SANTOS, R. S. S.; GARCIA, F. R. M.; OLIVEIRA, E. E.; BOTTON, M.; NAVA, D. E. *Drosophila suzukii* in Southern Neotropical Region: Current Status and Future**. PerspectivesNeotropical Entomology**, 2017b. .doi: 10.1007/s13744-017-0554-7.

ANDREAZZA, F.; HADDI, K.; OLIVEIRA, E. E.; FERREIRA, J. A. M. *Drosophila suzukii* (Diptera: Drosophilidae) Arrives at Minas Gerais State, a Main Strawberry Production Region in Brazil. **Florida Entomologist**, v. 99, n. 4, p. 796–798, dez. 2016. doi: 10.1653/024.099.0439.

BACCI, L.; LUPI, D.; SAVOLDELLI, S.; ROSSARO, B. A review of Spinosyns, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. **Journal of Entomological and Acarological Research**, v. 48, n. 1, p. 40, 28 abr. 2016. doi: 10.4081/jear.2016.5653.

BENÍTEZ, H. A.; PÜSCHEL, T. A. Modelando la Varianza de la Forma: Morfometría Geométrica Aplicaciones en Biología Evolutiva. **International Journal of Morphology**, v. 32, n. 3, p. 998–1008, set. 2014. doi: 10.4067/S0717-95022014000300041.

BERNARDI, D.; RIBEIRO, L.; ANDREAZZA, F.; NEITZKE, C.; OLIVEIRA, E. E.; BOTTON, M.; NAVA, D. E.; VENDRAMIM, J. D. Potential use of *Annona* by products to control *Drosophila suzukii* and toxicity to its parasitoid *Trichopria anastrephae*. **Industrial Crops and Products**, 2017. doi: 10.1016/j.indcrop.2017.09.004.

BONATO, O.; CHADOEUF, J. Arthropods life cycle and temperature: Beyond isomorphy hypothesis. **Journal of Thermal Biology**, v. 75, p. 1–6, jul. 2018. doi: 10.1016/j.jtherbio.2018.05.004.

BOŠKOVIĆ, D.; VUKOVIĆ, S.; LAZIĆ, S.; BASER, N.; ČULUM, D.; TEKIĆ, D.; ŽUNIĆ, A.; ŠUŠNJAR, A.; ŠUNJKA, D. Insecticidal Activity of Selected Essential Oils against *Drosophila suzukii* (Diptera: Drosophilidae). **Plants**, v. 12, n. 21, p. 3727, 30 out. 2023. doi: 10.3390/plants12213727.

BREVIK, K.; LINDSTRÖM, L.; MCKAY, S. D.; CHEN, Y. H. Transgenerational effects of insecticides — implications for rapid pest evolution in agroecosystems. **Current Opinion in**

Insect Science, v. 26, p. 34–40, abr. 2018. doi: 10.1016/j.cois.2017.12.007.

BUCKLEY, L. B. Temperature-sensitive development shapes insect phenological responses to climate change. **Current Opinion in Insect Science**, v. 52, p. 100897, ago. 2022. doi: 10.1016/j.cois.2022.100897.

BUJARRABAL, A.; SCHUMACHER, B. Hormesis running hot and cold. **Cell Cycle**, v. 15, n. 24, p. 3335–3336, 16 dez. 2016. doi: 10.1080/15384101.2016.1235859.

CAETANO, A. R. S.; CARDOSO, M. das G.; HADDI, K.; CAMPOLINA, G. A.; SOUZA, B. M.; LUNGUINHO, A.; SOUZA, L.; NELSON, D. L.; OLIVEIRA, J. E. *Rosmarinus officinalis* essential oil incorporated into nanoparticles as an efficient insecticide against *Drosophila suzukii* (Diptera: Drosophilidae). **Austral Entomology**, v. 61, n. 2, p. 265–272, 22 maio 2022. doi: 10.1111/aen.12592.

CAGÁŇ, Ľ.; APACSOVÁ FUSKOVÁ, M.; HLÁVKOVÁ, D.; SKOKOVÁ HABUŠTOVÁ, O. Essential Oils: Useful Tools in Storage-Pest Management. **Plants**, v. 11, n. 22, p. 3077, 13 nov. 2022. doi: 10.3390/plants11223077.

CALABRESE, E. J. Hormesis: Why it is important to toxicology and toxicologists. **Environmental Toxicology and Chemistry**, v. 27, n. 7, p. 1451, 2008. doi: 10.1897/07-541.1.

CALABRIA G, MACA J, BACHLI G, S. L. & P. M. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. **J Appl Entomol**, v. 136, p. 139–147, 2012.

CHOPRA, B.; DHINGRA, A. K. Natural products: A lead for drug discovery and development. **Phytotherapy Research**, v. 35, n. 9, p. 4660–4702, 13 set. 2021. doi: 10.1002/ptr.7099.

CLEMENTE, M.; FUSCO, G.; TONINA, L.; GIOMI, F. Temperature-induced phenotypic plasticity in the ovipositor of the invasive species *Drosophila suzukii*. **Journal of Thermal Biology**, v. 75, p. 62–68, jul. 2018. doi: 10.1016/j.jtherbio.2018.05.001.

COSSENTINE, J.; ROBERTSON, M.; BUITENHUIS, R. Impact of acquired entomopathogenic fungi on adult *Drosophila suzukii* survival and fecundity. **Biological Control**, v. 103, p. 129–137, dez. 2016. doi: 10.1016/j.biocontrol.2016.09.002.

COSTANTINI, D. Hormesis promotes evolutionary change. **Dose-Response**, v. 17, n. 2, p. 155932581984337, 1 abr. 2019. doi: 10.1177/1559325819843376.

CUTLER, G. C. Insects, Insecticides and Hormesis: Evidence and Considerations for Study. **Dose-Response**, v. 11, n. 2, p. dose-response.1, 1 abr. 2013. doi: 10.2203/dose-response.12-008.Cutler.

DAM, D.; MOLITOR, D.; BEYER, M. Natural compounds for controlling *Drosophila suzukii*. A review. **Agronomy for Sustainable Development**, v. 39, n. 6, p. 53, 13 dez. 2019. doi: 10.1007/s13593-019-0593-z.

DAVID, M. D. The potential of pro-insecticides for resistance management. **Pest Management Science**, v. 77, n. 8, p. 3631–3636, 29 ago. 2021. doi: 10.1002/ps.6369.

DE FIGUEIREDO, K. G.; DE PAIVA SILVA, G. T.; PASSOS, L. C.; ALVES, D. S.; BIONDI, A.; CARVALHO, G. A. Toxicity of *Cinnamomum* spp. essential oil to *Tuta absoluta* and to predatory mirid. **Journal of Pest Science**, 13 dez. 2023. doi: 10.1007/s10340-023-01719-0.

DE SOUZA, L.; CARDOSO, M. das G.; KONIG, I. F. M.; FERREIRA, V. R. F.; CAETANO, A. R. S.; CAMPOLINA, G. A.; HADDI, K. Toxicity, Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium verum* Essential Oil in *Drosophila suzukii*. **Agriculture**, v. 12, n. 10, p. 1667, 11 out. 2022. doi: 10.3390/agriculture12101667.

DEPRÁ, M.; POPPE, J. L.; SCHMITZ, H. J.; DE TONI, D. C.; VALENTE, V. L. S. The first records of the invasive pest *Drosophila suzukii* in the South American continent. **Journal of Pest Science**, v. 87, n. 3, p. 379–383, 16 set. 2014. doi: 10.1007/s10340-014-0591-5.

DISI, J. O.; VAN TIMMEREN, S.; GRESS, B.; ZALOM, F.; ISAACS, R.; SIAL, A. Insecticide residue longevity for on-site screening of *Drosophila suzukii* (Matsumura) resistance. **Pest Management Science**, 2020. doi: 10.1002/ps.5880.

DRIPPS, J. E.; DEAMICIS, C. V.; SPARKS, T. C.; CROUSE, G. D. Spinosad to spinetoram: evolution of the spinosyns. In: The 236th ACS National Meeting, **Anais**...2008.

DUQUE, J. E.; URBINA, D. L.; VESGA, L. C.; ORTIZ-RODRÍGUEZ, L. A.; VANEGAS, T. S.; STASHENKO, E. E.; MENDEZ-SANCHEZ, S. C. Insecticidal activity of essential oils from American native plants against *Aedes aegypti* (Diptera: Culicidae): an introduction to their possible mechanism of action. **Scientific Reports**, v. 13, n. 1, p. 2989, 20 fev. 2023. doi: 10.1038/s41598-023-30046-8.

EMILJANOWICZ, L. M.; RYAN, G. D.; LANGILLE, A.; NEWMAN, J. Development, Reproductive Output and Population Growth of the Fruit Fly Pest *Drosophila suzukii* (Diptera: Drosophilidae) on Artificial Diet. **Journal of Economic Entomology**, v. 107, n. 4, p. 1392–1398, 1 ago. 2014. doi: 10.1603/EC13504.

ENGLERT, C.; HERZ, A. Acceptability of *Drosophila suzukii* as prey for common predators occurring in cherries and berries. **Journal of Applied Entomology**, v. 143, n. 4, p. 387–396, 22 maio 2019. doi: 10.1111/jen.12613.

FAHRENTRAPP, J.; ROOSJEN, P.; GREEN, D. R.; GREGORY, B.; PANASSITI, B. color preferences of Spotted Wing Drosophila, *Drosophila suzukii*, under field conditions in various crops. **bioRxiv**, 2019.

FANNING, P. D.; GRIESHOP, M. J.; ISAACS, R. Efficacy of biopesticides on spotted wing drosophila, *Drosophila suzukii* Matsumura in fall red raspberries. **Journal of Applied Entomology**, v. 142, n. 1–2, p. 26–32, 24 Feb. 2018. doi: 10.1111/jen.12462.

FRAIMOUT, A.; JACQUEMART, P.; VILLARROEL, B.; APONTE, D. J.; DECAMPS, T.; HERREL, A.; CORNETTE, R.; DEBAT, V. Phenotypic plasticity of *Drosophila suzukii* wing to developmental temperature: implications for flight. **Journal of Experimental Biology**, v. 221, n. 13, p. jeb166868, 1 jul. 2018. doi: 10.1242/jeb.166868.

GABARRA, R.; RIUDAVETS, J.; RODRÍGUEZ, G. A.; PUJADE-VILLAR, J.; ARNÓ, J.

Prospects for the biological control of *Drosophila suzukii*. **BioControl**, v. 60, n. 3, p. 331–339, 14 jun. 2015. doi: 10.1007/s10526-014-9646-z.

GEISLER, F. C. S.; SANTOS, J.; HOLDEFER, D. R.; GARCIA, F. R. M. Primeiro registro *Drosophila suzukii* (MATSUMURA, 1931) (Diptera: Drosophilidae) para o estado do Paraná, Brasil e novos hospedeiros. **Revista de Ciências Ambientais**, v. 9, n. 2, 21 dez. 2015. doi: 10.18316/1981-8858.15.

GERDEMAN, B. S. ; TANIGOSHI, L. K. Biology and management of spotted wing drosophila, *Drosophila suzukii* (Matsumura) in small fruits in the Pacific Northwest. In: **Anais**...2010.

GEROFOTIS, C. D.; IOANNOU, C. S.; NAKAS, C. T.; PAPADOPOULOS, N. T. The odor of a plant metabolite affects life history traits in dietary restricted adult olive flies. **Scientific Reports**, v. 6, n. 1, p. 28540, jun. 2016. doi: 10.1038/srep28540.

GHALBANE, I.; ALAHYANE, H.; ABOUSSAID, H.; CHOUIKH, N.; COSTA, J.; ROMANE, A.; EL MESSOUSSI, S. Chemical Composition and Insecticidal Properties of Moroccan *Lavandula dentata* and *Lavandula stoechas* Essential Oils Against Mediterranean Fruit Fly, *Ceratitis capitata*. **Neotropical Entomology**, v. 51, n. 4, p. 628–636, 23 ago. 2022. doi: 10.1007/s13744-022-00975-6.

GIBERT, J.-M. La plasticité phénotypique chez les insectes. **Biologie Aujourd’hui**, v. 214, n. 1–2, p. 33–44, 10 ago. 2020. doi: 10.1051/jbio/2020005.

GIBERT, P.; DEBAT, V.; GHALAMBOR, C. K. Phenotypic plasticity, global change, and the speed of adaptive evolution. **Current Opinion in Insect Science**, v. 35, p. 34–40, out. 2019. doi: 10.1016/j.cois.2019.06.007.

GIUNTI, G.; BENELLI, G.; PALMERI, V.; LAUDANI, F.; RICUPERO, M.; RICCIARDI, R.; MAGGI, F.; LUCCHI, A.; GUEDES, R. N. C.; DESNEUX, N.; CAMPOLO, O. Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. **Biological Control**, v. 176, p. 105071, dez. 2022. doi: 10.1016/j.biocontrol.2022.105071.

GÖRÜR, G. The role of phenotypic plasticity in host race formation and sympatric speciation in phytophagous insects, particularly in aphids. **Turkish Journal of Zoology,** v. 24, n. 1, p. 63–68, 2000.

GRESS, B. E.; ZALOM, F. G. Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. **Pest Management Science**, v. 75, n. 5, p. 1270–1276, 4 maio 2019. doi: 10.1002/ps.5240.

GRESS, B. E.; ZALOM, F. G. Development and validation of a larval bioassay and selection protocol for insecticide resistance in *Drosophila suzukii*. **PLOS ONE**, v. 17, n. 6, p. e0270747, 29 jun. 2022. doi: 10.1371/journal.pone.0270747.

GUEDES, R. N. C.; RIX, R. R.; CUTLER, G. C. Pesticide-induced hormesis in arthropods: Towards biological systems. **Current Opinion in Toxicology**, v. 29, p. 43–50, mar. 2022. doi: 10.1016/j.cotox.2022.02.001.

HADDI, K.; TURCHEN, L. M.; VITERI JUMBO, L. O.; GUEDES, R. N.; PEREIRA, E. J.; AGUIAR, R. W.; OLIVEIRA, E. E. Rethinking biorational insecticides for pest management: unintended effects and consequences. **Pest Management Science**, v. 76, n. 7, p. 2286–2293, 23 jul. 2020. doi: 10.1002/ps.5837.

HAUSER, M. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. **Pest Management Science**, v. 67, n. 11, p. 1352–1357, nov. 2011. doi: 10.1002/ps.2265.

HAYE, T.; GIROD, P.; CUTHBERTSON, A. G. S.; WANG, X. G.; DAANE, K. M.; HOELMER, K. A.; BAROFFIO, C.; ZHANG, J. P.; DESNEUX, N. Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. **Journal of Pest Science**, v. 89, n. 3, p. 643–651, 8 jul. 2016. doi: 10.1007/s10340-016-0737-8.

HOFSTETTER, R.; FASSAUER, G. M.; LINK, A. Supercritical fluid extraction (SFE) of ketamine metabolites from dried urine and on-line quantification by supercritical fluid chromatography and single mass detection (on-line SFE–SFC–MS). **Journal of Chromatography B**, v. 1076, p. 77–83, fev. 2018. doi: 10.1016/j.jchromb.2018.01.024.

HUA, J.; JONES, D. K.; RELYEA, R. A. Induced Tolerance from a Sublethal Insecticide Leads to Cross-Tolerance to Other Insecticides. **Environmental Science & Technology**, v. 48, n. 7, p. 4078–4085, 1 abr. 2014. doi: 10.1021/es500278f.

ISMAN, M. B. Botanical Insecticides in the Twenty-First Century—Fulfilling Their Promise? **Annual Review of Entomology**, v. 65, n. 1, p. 233–249, 7 jan. 2020a. doi: 10.1146/annurev-ento-011019-025010.

ISMAN, M. B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. **Phytochemistry Reviews**, v. 19, n. 2, p. 235–241, 4 abr. 2020b. doi: 10.1007/s11101-019-09653-9.

IZAKMEHRI, K.; SABER, M.; MEHRVAR, A.; HASSANPOURAGHDAM, M. B.; VOJOUDI, S. Lethal and Sublethal Effects of Essential Oils from *Eucalyptus camaldulensis* and *Heracleum persicum* Against the Adults of *Callosobruchus Maculatus*. **Journal of Insect Science**, v. 13, n. 152, p. 1–10, dez. 2013. doi: 10.1673/031.013.15201.

JOHNSON, J. B. **Mechanism of action of natural and biorational insecticides**. 2021. Iowa State University, 2021. doi: 10.31274/etd-20210609-79.

KHAN, S.; UDDIN, M.; RIZWAN, M.; KHAN, W.; FAROOQ, M.; SATTAR SHAH, A.; SUBHAN, F.; AZIZ, F.; RAHMAN, K.; KHAN, A.; ALI, S.; MUHAMMAD, M. Mechanism of Insecticide Resistance in Insects/Pests. **Polish Journal of Environmental Studies**, v. 29, n. 3, p. 2023–2030, 31 mar. 2020. doi: 10.15244/pjoes/108513.

KANZAWA, T. Studies on a fruit fly, *Drosophila* sp. . **(in Japanese).**, v. 8, p. 302–303, 1934.

KANZAWA, T. **Research into the fruit-fly *Drosophila suzukii* Matsumura (preliminary report). Yamanashi Prefecture Agricultural Experiment Station, Kofu**. [s.l: s.n.].

KHANI, A.; ASGHARI, J. Insecticide Activity of Essential Oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* Against Two Stored Product Pests, the Flour Beetle, *Tribolium castaneum*, and the *Cowpea Weevi*l, Callosobruch. **Journal of Insect Science**, v. 12, n. 73, p. 1–10, jul. 2012. doi: 10.1673/031.012.7301.

KHURSHEED, A.; RATHER, M. A.; JAIN, V.; WANI, A. R.; RASOOL, S.; NAZIR, R.; MALIK, N. A.; MAJID, S. A. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. **Microbial Pathogenesis**, v. 173, p. 105854, dez. 2022. doi: 10.1016/j.micpath.2022.105854.

KILANI-MORAKCHI, S.; MORAKCHI-GOUDJIL, H.; SIFI, K. Azadirachtin-Based Insecticide: Overview, Risk Assessments, and Future Directions. **Frontiers in Agronomy**, v. 3, 20 jul. 2021. doi: 10.3389/fagro.2021.676208.

KIRUTHIKA, S.; VISHALI, S. Industrial Application of Essential Oils. In: **Essential Oils**. [s.l.] Wiley, 2023. p. 49–67. 2023.

KLINGENBERG, C. P. Morpho J: an integrated software package for geometric morphometrics. **Molecular Ecology Resources**, v. 11, n. 2, p. 353–357, 5 mar. 2011. doi: 10.1111/j.1755-0998.2010.02924.x.

LAWING, A. M.; POLLY, P. D. Geometric morphometrics: recent applications to the study of evolution and development. **Journal of Zoology**, v. 280, n. 1, p. 1–7, 17 jan. 2010. doi: 10.1111/j.1469-7998.2009.00620.x.

LEE, J. C.; WANG, X.; DAANE, K. M.; HOELMER, K. A.; ISAACS, R.; SIAL, A. A.; WALTON, V. M. Biological Control of Spotted-Wing *Drosophila* (Diptera: Drosophilidae) Current and Pending Tactics. **Journal of Integrated Pest Management**, v. 10, n. 1, 1 jan. 2019. doi: 10.1093/jipm/pmz012.

LIBS, E.; SALIM, E. Formulation of essential oil pesticides technology and their application. **Agricultural Research & Technology Open Access Journal**, v. 9, n. 2, p. 555759, 2017.

LITTLE, C. M.; CHAPMAN, T. W.; HILLIER, N. K. Plasticity Is Key to Success of *Drosophila suzukii* (Diptera: Drosophilidae) Invasion. **Journal of Insect Science**, v. 20, n. 3, 1 maio 2020. doi: 10.1093/jisesa/ieaa034.

LU, C.; HUNG, Y.-T.; CHENG, Q. A Review of Sub-lethal Neonicotinoid Insecticides Exposure and Effects on Pollinators. **Current Pollution Reports**, v. 6, n. 2, p. 137–151, 19 jun. 2020. doi: 10.1007/s40726-020-00142-8.

MADEMTZOGLOU, D.; PAVLIDOU, T.; BAZIOTI, M.; KOUTSONIKOU, C.; LIOULIA, E.; AKMOUTSOU, P.; DROSOPOULOU, E.; VOKOU, D.; MAVRAGANI-TSIPIDOU, P. Assessment of the genotoxic potential of essential oil constituents by the *Drosophila* wing spot test. **Flavour and Fragrance Journal**, v. 28, n. 3, p. 188–194, 22 maio 2013. doi: 10.1002/ffj.3157.

MALLARD, F.; NOLTE, V.; SCHLÖTTERER, C. The Evolution of Phenotypic Plasticity in Response to Temperature Stress. **Genome Biology and Evolution**, v. 12, n. 12, p. 2429–2440, 6 dez. 2020. doi: 10.1093/gbe/evaa206.

MARCHIORI, C.; BARBARESCO, L. Occurrence of *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) as a parasitoid of *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae) in Brazil. **Brazilian Journal of Biology**, v. 67, n. 3, p. 577–578, ago. 2007. doi: 10.1590/S1519-69842007000300025.

MERTZ, F. P.; YAO, R. C. Saccharopolyspora spinosa sp. nov. Isolated from Soil Collected in a Sugar Mill Rum Still. **International Journal of Systematic Bacteriology**, v. 40, n. 1, p. 34–39, 1 jan. 1990. doi: 10.1099/00207713-40-1-34.

MOCZEK, A. P. Phenotypic plasticity and diversity in insects. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 365, n. 1540, p. 593–603, 27 Feb. 2010. doi: 10.1098/rstb.2009.0263.

MOHARRAMIPOUR, S.; NEGAHBAN, M. Plant Essential Oils and Pest Management. In: **Basic and Applied Aspects of Biopesticides**. New Delhi: Springer India, 2014. p. 129–153. 2014.

MOSSA, A.-T. H. Green Pesticides: Essential Oils as Biopesticides in Insect-pest Management. **Journal of Environmental Science and Technology**, v. 9, n. 5, p. 354–378, 15 ago. 2016. doi: 10.3923/jest.2016.354.378.

NAVA, DORI EDSON; BOTTON, M., BERNARDI, D., ANDREAZZA, F. & BARONIO, C. A. Bioecologia, monitoramento e controle de *Drosophila suzukii* na cultura de morangueiro. **Embrapa Agrobiologia - Documentos (INFOTECA-E)**, p. 30, 2015.

PAMELA, G. Pesticidal natural products – status and future potential. **Pest Management Science**, v. 75, n. 9, p. 2325–2340, 15 set. 2019. doi: 10.1002/ps.5433.

PANDIAN, S.; RAMESH, M. Development of Pesticide Resistance in Pests. In: **Pesticides in Crop Production**. [s.l.] Wiley, 2020. p. 1–13. 2020.

PAPANASTASIOU, S. A.; BALI, E.-M. D.; IOANNOU, C. S.; PAPACHRISTOS, D. P.; ZARPAS, K. D.; PAPADOPOULOS, N. T. Toxic and hormetic-like effects of three components of citrus essential oils on adult Mediterranean fruit flies (*Ceratitis capitata*). **PLOS ONE**, v. 12, n. 5, p. e0177837, 16 maio 2017. doi: 10.1371/journal.pone.0177837.

PAVELA, R.; MAGGI, F.; MAZZARA, E.; TORRESI, J.; CIANFAGLIONE, K.; BENELLI, G.; CANALE, A. Prolonged sublethal effects of essential oils from non-wood parts of nine conifers on key insect pests and vectors. **Industrial Crops and Products**, v. 168, p. 113590, set. 2021. doi: 10.1016/j.indcrop.2021.113590.

PÉREZ, E. R.; PAREDES, J. P. A.; PAPIC, C. C.; PEÑA, F. A. Manual de reconocimiento de estados de desarrollo de *Drosophila suzukii* (Diptera: Drosophilidae) y otras especies del género, comunes en el sur de Chile. . 2019, p. 78.

PERRY, T.; MCKENZIE, J. A.; BATTERHAM, P. A D α 6 knockout strain of *Drosophila melanogaster* confers a high level of resistance to spinosad. **Insect Biochemistry and Molecular Biology**, 2007. doi: 10.1016/j.ibmb.2006.11.009.

PINEDA, M.; ALVES, E. L. de A.; ANTUNES, J. A.; CARVALHO, V. de C.; HADDI, K. Low Concentrations of *Eucalyptus* Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. **Agriculture**, v. 13, n. 2, p. 404, 9 fev. 2023. doi: 10.3390/agriculture13020404.

RENKEMA, J. M.; CUTHBERTSON, A. G. S. Impact of multiple natural enemies on immature *Drosophila suzukii* in strawberries and blueberries. **BioControl**, v. 63, n. 5, p. 719–728, 3 out. 2018. doi: 10.1007/s10526-018-9874-8.

RENKEMA, J. M.; WRIGHT, D.; BUITENHUIS, R.; HALLETT, R. H. Plant essential oils and potassium metabisulfite as repellents for *Drosophila suzukii* (Diptera: Drosophilidae). **Scientific Reports**, v. 6, n. 1, p. 21432, 19 fev. 2016. doi: 10.1038/srep21432.

REYES, M.; ROCHA, K.; ALARCÓN, L.; SIEGWART, M.; SAUPHANOR, B. Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad. **Pesticide Biochemistry and Physiology**, 2012. doi: 10.1016/j.pestbp.2011.10.008.

RHOUMA, A.; HAJJI-HEDFI, L.; BOUSSELMA, A.; MATROOD, A. A. A. Essential Oils: An Ecofriendly approach for plant pest and disease management. **International Journal of Plant & Soil Science**, v. 35, n. 8, p. 80–89, 3 abr. 2023. doi: 10.9734/ijpss/2023/v35i82883.

RIX, R. R.; GUEDES, R. N. C.; CHRISTOPHER CUTLER, G. Hormesis dose–response contaminant-induced hormesis in animals. **Current Opinion in Toxicology**, v. 30, p. 100336, jun. 2022. doi: 10.1016/j.cotox.2022.02.009.

ROHLF, F. J.; SLICE, D. Extensions of the Procrustes Method for the Optimal Superimposition of Landmarks. **Systematic Zoology**, v. 39, n. 1, p. 40, mar. 1990. doi: 10.2307/2992207.

ROSAS-GARCÍA, N. M.; TORRES-ORTEGA, J. A.; VILLEGAS-MENDOZA, J. M. Lethal and Sublethal Effects of Commercial Essential Oils on the Life Cycle of *Spodoptera exigua* (Hübner)1. **Southwestern Entomologist**, v. 46, n. 3, 29 set. 2021. doi: 10.3958/059.046.0303.

SÁNCHEZ-RAMOS, I.; FERNÁNDEZ, C. E.; GONZÁLEZ-NÚÑEZ, M. Laboratory evaluation of insect growth regulators against the spotted wing drosophila, *Drosophila suzukii*. **Journal of Pest Science**, 21 jun. 2023. doi: 10.1007/s10340-023-01648-y.

SCHLESENER, D. C. H. WOLLMANN, J.; NUNES, A. M.; CORDEIRO, J.; GOTTSCHALK, M. S.; GARCIA, F. R. M. *Drosophila suzukii*: nova praga para a fruticultura brasileira. **O Biológico**, v. 77, n. 1, p. 47–54, 2015.

SCHLESENER, D. C. H.; WOLLMANN, J.; PAZINI, J. D. B.; PADILHA, A. C.; GRÜTZMACHER, A. D.; GARCIA, F. R. M. Insecticide Toxicity to *Drosophila suzukii* (Diptera: Drosophilidae) parasitoids: *Trichopria anastrephae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae). **Journal of Economic Entomology**, 2019. doi: 10.1093/jee/toz033.

SCHLESENER, H. D.; WOLLMANN, J.; KRÜGER, A. P.; NUNES, A. M.; BERNARDI, D.; GARCIA, F. R. M. Biology and fertility life table of *Drosophila suzukii* on artificial diets. **Entomologia Experimentalis et Applicata**, 2018. doi: 10.1111/eea.12736.

SCHÖNEBERG, T.; ARSENAULT-BENOIT, A.; TAYLOR, C. M.; BUTLER, B. R.; DALTON, D. T.; WALTON, V. M.; PETRAN, A.; ROGERS, M. A.; DIEPENBROCK, L. M.; BURRACK, H. J.; LEACH, H.; VAN TIMMEREN, S.; FANNING, P. D.; ISAACS, R.; GRESS, B. E.; BOLDA, M. P.; ZALOM, F. G.; ROUBOS, C. R.; EVANS, R. K.; SIAL, A. A.; HAMBY, K. A. Pruning of small fruit crops can affect habitat suitability for *Drosophila suzukii*. **Agriculture, Ecosystems & Environment**, v. 294, p. 106860, jun. 2020. doi: 10.1016/j.agee.2020.106860.

SHAW, B.; HEMER, S.; CANNON, M. F. L.; ROGAI, F.; FOUNTAIN, M. T. Insecticide Control of *Drosophila suzukii* in Commercial Sweet Cherry Crops under Cladding. **Insects**, v. 10, n. 7, p. 196, 4 jul. 2019. doi: 10.3390/insects10070196.

SHAWER, R. Chemical Control of *Drosophila suzukii*. In: *Drosophila suzukii* Management. Cham: Springer International Publishing, 2020. p. 133–142. 2020.

SHIRVANI, Z.; ALLAHYARI, H.; GOLPAYEGANI, A. Z.; JAHROMI, K. T.; DÖKER, I. Influence of sub-lethal exposure to *Rosmarinus officinalis* L. (Lamiaceae) essential oil on demographic parameters of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiideae). **International Journal of Acarology**, v. 49, n. 5–6, p. 270–276, 18 ago. 2023. doi: 10.1080/01647954.2023.2244505.

SIAL, A. A.; ROUBOS, C. R.; GAUTAM, B. K.; FANNING, P. D.; VAN TIMMEREN, S.; SPIES, J.; PETRAN, A.; ROGERS, M. A.; LIBURD, O. E.; LITTLE, B. A.; CURRY, S.; ISAACS, R. Evaluation of organic insecticides for management of spotted-wing drosophila (*Drosophila suzukii*) in berry crops. **Journal of Applied Entomology**, v. 143, n. 6, p. 593–608, 28 jul. 2019. doi: 10.1111/jen.12629.

SILVA, A. P. N.; CHAGAS, C. F.; DE ANDRADE ALVES, E. L.; DE CASTRO CARVALHO, V.; HADDI, K. Temperature effects on the hormetic response of *Myzus persicae* after sublethal exposure to insecticides. CABI Agriculture and Bioscience, v. 5, n. 1, p. 5, 10 jan. 2024. doi: 10.1186/s43170-024-00213-6.

SILVA, S. M.; HADDI, K.; VITERI JUMBO, L. O.; OLIVEIRA, E. E. Progeny of the maize weevil, *Sitophilus zeamais*, is affected by parental exposure to clove and *Cinnamon* essential oils. **Entomologia Experimentalis et Applicata**, v. 163, n. 2, p. 220–228, maio 2017a. doi: 10.1111/eea.12559.

SILVA, W. M.; BERGER, M.; BASS, C.; WILLIAMSON, M.; MOURA, D. M. N.; RIBEIRO, L. M. S.; SIQUEIRA, H. A. A. Mutation (G275E) of the nicotinic acetylcholine receptor α6 subunit is associated with high levels of resistance to spinosyns in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). **Pesticide Biochemistry and Physiology**, 2016. doi: 10.1016/j.pestbp.2016.02.006.

SIOMAVA, N.; WIMMER, E. A.; POSNIEN, N. Size relationships of different body parts in the three dipteran species *Drosophila melanogaster*, *Ceratitis capitata,* and, *Musca domestica*. **Development Genes and Evolution**, v. 226, n. 3, p. 245–256, 26 jun. 2016. doi: 10.1007/s00427-016-0543-6.

SOUZA, M. T. de; SOUZA, M. T. de; MORAIS, M. C.; OLIVEIRA, D. da C.; MELO, D. J.

de; FIGUEIREDO, L.; ZARBIN, P. H. G.; ZAWADNEAK, M. A. C.; BERNARDI, D. Essential Oils as a Source of Ecofriendly Insecticides for *Drosophila suzukii* (Diptera: Drosophilidae) and Their Potential Non-Target Effects. **Molecules**, v. 27, n. 19, p. 6215, 21 set. 2022. doi: 10.3390/molecules27196215.

SWARTZ, W.; LORENTZ, S.; PAPADAKIS, S.; HUANG, P.; SMITH, A.; DEGLAU, D.; YU, Y.; REILLY, S.; REILLY, N.; ANDERSON, D. RAVAN: CubeSat Demonstration for Multi-Point Earth Radiation Budget Measurements. **Remote Sensing**, v. 11, n. 7, p. 796, 3 abr. 2019. doi: 10.3390/rs11070796.

TAIT, G.; KAISER, C.; STACCONI, R.; DALTON, D. T.; ANFORA, G.; WALTON, V. M. A food-grade gum as a management tool for *Drosophila suzukii*. **Bulletin of Insectology**, v. 71, n. 2, p. 295–307, 2018. doi: http://hdl.handle.net/10449/51742.

TAIT, G.; MERMER, S.; STOCKTON, D.; LEE, J.; AVOSANI, S.; ABRIEUX, A.; ANFORA, G.; BEERS, E.; BIONDI, A.; BURRACK, H.; CHA, D.; CHIU, J. C.; CHOI, M.-Y.; CLOONAN, K.; CRAVA, C. M.; DAANE, K. M.; DALTON, D. T.; DIEPENBROCK, L.; FANNING, P.; GANJISAFFAR, F.; GÓMEZ, M. I.; GUT, L.; GRASSI, A.; HAMBY, K.; HOELMER, K. A.; IORIATTI, C.; ISAACS, R.; KLICK, J.; KRAFT, L.; LOEB, G.; ROSSI-STACCONI, M. V.; NIERI, R.; PFAB, F.; PUPPATO, S.; RENDON, D.; RENKEMA, J.; RODRIGUEZ-SAONA, C.; ROGERS, M.; SASSÙ, F.; SCHÖNEBERG, T.; SCOTT, M. J.; SEAGRAVES, M.; SIAL, A.; VAN TIMMEREN, S.; WALLINGFORD, A.; WANG, X.; YEH, D. A.; ZALOM, F. G.; WALTON, V. M. *Drosophila suzukii* (Diptera: Drosophilidae): A Decade of Research Towards a Sustainable Integrated Pest Management Program. **Journal of Economic Entomology**, v. 114, n. 5, p. 1950–1974, 13 out. 2021. doi: 10.1093/jee/toab158.

TATSUTA, H.; TAKAHASHI, K. H.; SAKAMAKI, Y. Geometric morphometrics in entomology: Basics and applications. **Entomological Science**, v. 21, n. 2, p. 164–184, 26 jun. 2018. doi: 10.1111/ens.12293.

TOMINAGA, Y. Studies on breeding of pyrethrum flowers. **Bulletin of the Hiroshima Agricultural College (Japan)**, v. 7, n. 1, 1982.

TRAN, A. K.; HUTCHISON, W. D.; ASPLEN, M. K. Morphometric criteria to differentiate *Drosophila suzukii* (Diptera: Drosophilidae) seasonal morphs. **PLOS ONE**, v. 15, n. 2, p. e0228780, 6 fev. 2020. doi: 10.1371/journal.pone.0228780.

TRIPATHI, A.K., S.; UPADHYAY, M.; BHUIYAN, B.; BHATTACHARYA, P. B. A review on prospects of essential oils as biopesticide in insect-pest management. **Journal of Pharmacognosy and Phytotherapy**, v. 51, 2009.

VAN TIMMEREN, S.; ISAACS, R. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. **Crop Protection**, v. 54, p. 126–133, dez. 2013. doi: 10.1016/j.cropro.2013.08.003.

VARÓN-GONZÁLEZ, C.; FRAIMOUT, A.; DELAPRÉ, A.; DEBAT, V.; CORNETTE, R. Limited thermal plasticity and geographical divergence in the ovipositor of *Drosophila suzukii*. **Royal Society Open Science**, v. 7, n. 1, p. 191577, 22 jan. 2020. doi: 10.1098/rsos.191577.

VILLALOBOS-LEIVA, A.; BENÍTEZ, H. A. Geometric morphometric and its new applications in ecology and evolutionary biology. Part 2. **International Journal of Morphology**, v. 38, n. 6, p. 1818–1836, 2020.

WATSON, G. B.; CHOUINARD, S. W.; COOK, K. R.; GENG, C.; GIFFORD, J. M.; GUSTAFSON, G. D.; HASLER, J. M.; LARRINUA, I. M.; LETHERER, T. J.; MITCHELL, J. C. A spinosyn-sensitive Drosophila melanogaster nicotinic acetylcholine receptor identified through chemically induced target site resistance, resistance gene identification, and heterologous expression. **Insect Biochemistry and Molecular Biology**, v. 40, n. 5, p. 376–384, maio 2010. doi: 10.1016/j.ibmb.2009.11.004.

WINKLER, A.; JUNG, J.; KLEINHENZ, B.; RACCA, P. Estimating temperature effects on *Drosophila suzukii,* life cycle parameters. **Agricultural and Forest Entomology**, v. 23, n. 3, p. 361–377, 20 ago. 2021. doi: 10.1111/afe.12438.

WISE, J. C.; VANDERPOPPEN, R.; VANDERVOORT, C.; O'DONNELL, C.; ISAACS, R. Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. **The Canadian Entomologist**, v. 147, n. 1, p. 109–117, 6 fev. 2015. doi: 10.4039/tce.2014.36.

YEE, W. L.; ALSTON, D. G. Effects of Spinosad, spinosad Bait, and Chloronicotinyl Insecticides on Mortality and Control of Adult and Larval Western Cherry Fruit Fly (Diptera: Tephritidae). **Journal of Economic Entomology**, 2009. doi: 10.1603/0022-0493-99.5.1722.

ZENI, V.; BENELLI, G.; CAMPOLO, O.; GIUNTI, G.; PALMERI, V.; MAGGI, F.; RIZZO, R.; LO VERDE, G.; LUCCHI, A.; CANALE, A. Toxics or Lures? Biological and Behavioral Effects of Plant Essential Oils on Tephritidae Fruit Flies. **Molecules**, v. 26, n. 19, p. 5898, 29 set. 2021. doi: 10.3390/molecules26195898.

ZÚÑIGA-REINOSO, Á.; BENÍTEZ, H. A. The overrated use of the morphological cryptic species concept: An example with *Nyctelia darkbeetles* (Coleoptera: Tenebrionidae) using geometric morphometrics. **Zoologischer Anzeiger - A Journal of Comparative Zoology**, v. 255, p. 47–53, mar. 2015. doi: 10.1016/j.jcz.2015.01.004.

SECOND PART: ARTICLES

ARTICLE I

Low concentrations of eucalyptus essential oil induce age, sex, and mating status-dependent stimulatory responses in *Drosophila suzukii*

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Published Article

https://doi.org/10.3390/agriculture13020404

Agriculture by MDPI.

Submission received: 10 January 2023 / Revised: 2 February 2023

Accepted: 7 February 2023 / Published: 9 February 2023

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 are largely focusing on their activity on targeted insect pests while overlooking their potential unintended effects on insect biological and reproductive traits, especially during sublethal exposures. Here, we first determined the toxicity of eucalyptus *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 four newly emerged virgin flies' couple 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.

Introduction

In 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 [5][2]. Such exposure can affect different aspects of exposed insects' behavioral, biological, and reproductive traits and when it leads to stimulatory responses, it is termed 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 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 insecticides, although they are generally natural mixtures of several molecules with many different modes of action. Similarly 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 based mainly on 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 (Myrtaceae family), 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*), 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 flies as a model to evaluate not only the lethal 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.

Material and Methods

Drosophila suzukii rearing

The stock colony of *D. suzukii*, used in the experiments is 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 are maintained in plastic cages (10cm height x 25cm diameter), using an artificial diet and following previously described methods [27,28]. The cages are kept under controlled conditions of temperature (T: 23 ± 2^{o}C), relative humidity (RH: 60 ± 5%) and scotophase (12H).

Eucalyptus essential oil

The EO of eucalyptus *Eucalyptus globulus* Labill (Myrtales: Myrtaceae) was purchased commercially in sealed amber bottles with a volume of 10 mL from WNF Indústria e Comercio Ltda[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.

Toxicity assessment of Eucalyptus EO against *Drosophila suzukii*

The toxicity of eucalyptus EO was evaluated using the exposure method described in protocol No.26 of the Insecticide Resistance Action Committee (IRAC) [34] 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 to the final concentration the corresponding EO volumes in 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, the 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±2ºC, 60±5% relative humidity (RH), and 12H scotophase. Mortality was evaluated after 48 hours of exposure. Flies were considered dead if they did not show any movement even after being podded with a fine brush.

Exposure of old mated adults to eucalyptus EO low doses

Once the dose-response curve of eucalyptus EO was determined, the concentrations CL_0 (control water + DMSO), $CL_{5,}$ and CL_{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 hours, mortality was evaluated and the survivors were transferred to new containers with sugar water solution (20% w/v). After 24 hours, 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 new glass with diet. For each concentration, 10 pupae were taken to estimate their mass. The daily number of emerged flies for each treatment was counted, and the body weight of newly emerged (less than 1 day) females (50) and males (50) was also determined.

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 that emerged, 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 ($p \leq 0.05$).

Results

Toxicity bioassays

The mortality levels obtained in the concentration-mortality bioassay were satisfactorily described by the probit model with goodness-of-fit test exhibiting low χ2-value ($\chi^2 = 1.10$) and p-value ($p = 0.98$) higher than 0.05. Mortality in the control group did not exceed 10%. The tested *E. globulus* EO has 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).

Effects of low concentrations of eucalyptus EO on the biological and reproductive traits of old mated flies

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 females (Log-Rank test, $\chi^2 = 1.93$; $df = 2$; $p = 0.39$; Figure 1A) or the males (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.

Parental flies’ fertility

Females’ fertility was assessed by counting the number of emerged females, males and the total (females + males) adults at 8 and 16 days as well as the aggregate of the numbers of adults who emerged during the whole experiment (day 8 + day 16; Figure 2). Both at 8 and 16 days, no statistical significant differences were found for the total number of males and females (8 days: F= 2.262; df =2; $p = 0.147$ and 16 days: F= 2.182; df =2; $p = 0.252$; Figure 2A), the number of females (8 days: F= 3.462; df =2; $p = 0.065$ and 16 days: F= 0.825; df =2; $p = 0.462$;

Figure 2B) and males (8 days: F= 0.970; *df* =2; *p* = 0.407 and 16 days: F= 3.327; *df* =2; *p* = 0.071; Figure 2C) of emerged flies between the treatments exposed to CL_5 and CL_{20} of essential oil and the unexposed control (CL_0). Interestingly, when the aggregate of the numbers of adults emerged during the whole experiment (day 8 + day 16) were compared, significant statistical differences were found between 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.

Progeny pupal and flies' body mass

The analysis of variance results showed that there was 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 1). Such differences were not carried over to either 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).

Effects of low concentrations of eucalyptus EO on the biological and reproductive traits of newly emerged virgin adults

Parental flies' longevity

Significant differences were found between the four treatments for the longevity of the newly emerged and virgin females (Log-Rank test, χ^2 = 70.81; *df* = 3; *p* < 0.001; Figure 3A) and males (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 the 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.

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 (8 days: F= 0.764; *df* = 3; *p* = 0.531 and 16 days F= 2.678; *df* = 3; *p* = 0.082) but presented contrasting differences in the 3rd (H= 8.758; *df* = 3; *p* = 0.033) and 4th (F= 7.054; *df* = 3; *p* = 0.003) weeks (Figure 4 A).

The untreated couples (♂NT x ♀NT) showed higher females (F= 2.328; *df* = 3; *p* = 0.113; Figure 4 B) and males (F= 2.453; *df* = 3; *p* = 0.101; Figure 4 C) emergences in the 2nd 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 due mainly 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 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).

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 males (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 equally to the controls (♂NT x ♀NT) and produced the highest numbers of flies.

Progeny flies' body mass

The body mass of the adults flies of the progeny did not differ between the four treatments neither for females (H = 2.59; *df* = 3; *p* = 0.47) nor males (F = 1.99; *df* = 2; *p* = 0.58) emerged flies (Supplementary figure S1).

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*. Besides our findings indicating 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 pupae 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 µl.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 repellency[34] activities against this insect.

Essential oils or their 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 plants 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 extracts 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 (CL_{20}), the total number of their progeny was higher compared to untreated flies while their longevity was not impacted. On the contrary, when virgin unmated flies were exposed to the low dose of the EO, only the 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 doses was based on the established concentration-response curve where 5 to 7 old unsexed individuals were used. Such experimental conditions would explain, although partially, the sex- and age-dependent effects. *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 trade-offs 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 in juvenile hormone levels [50,51] or a shift in resource allocation [51] when they occur between longevity and reproduction.

Although 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 a mechanism still needs to be further investigated and its validity proved in phytochemical-induced stimulatory responses in insects.

Conclusion

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 exposure to a 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 to insect pests is critical to determine the implications of the implementation and sustainability of essential oils within insect pest management programs.

Acknowledgments

CRediT authorship contribution statement

M.P: Methodology, Investigation, Formal analysis, writing – original draft; **E.L.A.A**: Investigation, data curation; **J.A.A**: Investigation, **V.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.

Acknowledgments: This work was supported by the National Council of Scientific and Techno-logical Development (CNPq), Minas Gerais State Foundation for Research Aid (FAPEMIG, APQ-02230-21) and Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Capes, Brazil) (Finance code 001).

Declaration 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.

Funding: This research was funded by National Council of Scientific and Technological Development (CNPq), Minas Gerais State Foundation for Research Aid (FAPEMIG, APQ-02230-21) and Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Capes, Brazil) (Finance code 001).

References

1. Benelli, G.; Rizzo, R.; Zeni, V.; Govigli, A.; Samková, A.; Sinacori, M.; Lo Verde, G.; Pavela, R.; Cappellacci, L.; Petrelli, R.; et al. *Carlina Acaulis* and *Trachyspermum Ammi* Essential Oils Formulated in Protein Baits Are Highly Toxic and Reduce Aggressiveness in the Medfly, *Ceratitis* Capitata. *Ind. Crops Prod.* **2021**, *161*, 113191, doi:10.1016/j.indcrop.2020.113191.

2. Haddi, K.; Turchen, L.M.; Viteri Jumbo, L.O.; Guedes, R.N.; Pereira, E.J.; Aguiar, R.W.; Oliveira, E.E. Rethinking Biorational Insecticides for Pest Management: Unintended Effects and Consequences. *Pest Manag. Sci.* **2020**, *76*, 2286–2293, doi:10.1002/ps.5837.

3. Isman, M.B. Commercial Development of Plant Essential Oils and Their Constituents as Active Ingredients in Bioinsecticides. *Phytochem. Rev.* **2020**, *19*, 235–241, doi:10.1007/s11101-019-09653-9.

4. P. Ferreira, T.; Haddi, K.; F. T. Corrêa, R.; Zapata, V.L.B.; Piau, T.B.; Souza, L.F.N.; Santos, S.-M.G.; Oliveira, E.E.; Jumbo, L.O. V.; Ribeiro, B.M.; et al. Prolonged Mosquitocidal Activity of *Siparuna Guianensis* Essential Oil Encapsulated in Chitosan Nanoparticles. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007624, doi:10.1371/journal.pntd.0007624.

5. Rix, R.R.; Guedes, R.N.C.; Christopher Cutler, G. Hormesis Dose–Response Contaminant-Induced Hormesis in Animals. *Curr. Opin. Toxicol.* **2022**, *30*, 100336, doi:10.1016/j.cotox.2022.02.009.

6. Cutler, G.C.; Amichot, M.; Benelli, G.; Guedes, R.N.C.; Qu, Y.; Rix, R.R.; Ullah, F.; Desneux, N. Hormesis and Insects: Effects and Interactions in Agroecosystems. *Sci. Total Environ.* **2022**, *825*, 153899, doi:10.1016/j.scitotenv.2022.153899.

7. Guedes, R.N.C.; Rix, R.R.; Cutler, G.C. Pesticide-Induced Hormesis in Arthropods: Towards Biological Systems. *Curr. Opin. Toxicol.* **2022**, *29*, 43–50, doi:10.1016/j.cotox.2022.02.001.

8. Haddi, K.; Oliveira, E.E.; Faroni, L.R.A.; Guedes, D.C.; Miranda, N.N.S. Sublethal Exposure to Clove and Cinnamon Essential Oils Induces Hormetic-Like Responses and Disturbs Behavioral and Respiratory Responses in *Sitophilus Zeamais* (Coleoptera:

Curculionidae). *J. Econ. Entomol.* **2015**, *108*, 2815–2822, doi:10.1093/jee/tov255.

9. Leonov, A.; Arlia-Ciommo, A.; Piano, A.; Svistkova, V.; Lutchman, V.; Medkour, Y.; Titorenko, V. Longevity Extension by Phytochemicals. *Molecules* **2015**, *20*, 6544–6572, doi:10.3390/molecules20046544.

10. Gerofotis, C.D.; Ioannou, C.S.; Nakas, C.T.; Papadopoulos, N.T. The Odor of a Plant Metabolite Affects Life History Traits in Dietary Restricted Adult Olive Flies. *Sci. Rep.* **2016**, *6*, 28540, doi:10.1038/srep28540.

11. Papanastasiou, S.A.; Bali, E.-M.D.; Ioannou, C.S.; Papachristos, D.P.; Zarpas, K.D.; Papadopoulos, N.T. Toxic and Hormetic-like Effects of Three Components of Citrus Essential Oils on Adult Mediterranean Fruit Flies (*Ceratitis Capitata*). *PLoS One* **2017**, *12*, e0177837, doi:10.1371/journal.pone.0177837.

12. Asplen, M.K., G. Anfora, A. Biondi, D.S. Choi, D. Chu, K.M. Daane, P. Gilbert, A.P.; Gutierrez, K.A. Hoelmer, W.D. Hutchison, R. Isaacs, Z.L. Jiang, Z. Kárpáti, M.T. Kimura, M. Pascual, C.R.; Philips, C. Plantamps, L. Ponti, G. Vétek, H. Vogt, V.M. Walton, Y. Yu, L. Zappalà, and N.D. Invasion Biology of Spotted Wing *Drosophila* (*Drosophila Suzukii*): A Global Perspective and Future Priorities. *J. Pest Sci. (2004).* **2015**, *88*, 469–494.

13. Lee, J.C.; Bruck, D.J.; Dreves, A.J.; Ioriatti, C.; Vogt, H.; Baufeld, P. In Focus: Spotted Wing Drosophila, *Drosophila Suzukii*, across Perspectives. *Pest Manag. Sci.* 2011.

14. Hamby, K.A.; E. Bellamy, D.; Chiu, J.C.; Lee, J.C.; Walton, V.M.; Wiman, N.G.; York, R.M.; Biondi, A. Biotic and Abiotic Factors Impacting Development, Behavior, Phenology, and Reproductive Biology of *Drosophila Suzukii*. *J. Pest Sci. (2004).* **2016**, *89*, 605–619, doi:10.1007/s10340-016-0756-5.

15. Deprá, M.; Poppe, J.L.; Schmitz, H.J.; De Toni, D.C.; Valente, V.L.S. The First Records of the Invasive Pest *Drosophila Suzukii* in the South American Continent. *J. Pest Sci. (2004).* **2014**, *87*, 379–383, doi:10.1007/s10340-014-0591-5.

16. Andreazza, F.; Haddi, K.; Oliveira, E.E.; Ferreira, J.A.M. *Drosophila Suzukii* (Diptera: Drosophilidae) Arrives at Minas Gerais State, a Main Strawberry Production Region in Brazil. *Florida Entomol.* **2016**, *99*, 796–798, doi:10.1653/024.099.0439.

17. Cahenzli, F.; Strack, T.; Daniel, C. Screening of 25 Different Natural Crop Protection Products against *Drosophila Suzukii*. *J. Appl. Entomol.* **2018**, *142*, 563–577, doi:10.1111/jen.12510.

18. Dam, D.; Molitor, D.; Beyer, M. Natural Compounds for Controlling *Drosophila Suzukii*. A Review. *Agron. Sustain. Dev.* **2019**, *39*, 53, doi:10.1007/s13593-019-0593-z.

19. Eben, A.; Sporer, F.; Vogt, H.; Wetterauer, P.; Wink, M. Search for Alternative Control Strategies of *Drosophila Suzukii* (Diptera: Drosophilidae): Laboratory Assays Using Volatile Natural Plant Compounds. *Insects* **2020**, *11*, 811, doi:10.3390/insects11110811.

20. Gullickson, M.; Flavin Hodge, C.; Hegeman, A.; Rogers, M. Deterrent Effects of Essential Oils on Spotted-Wing Drosophila (*Drosophila Suzukii*): Implications for Organic Management in Berry Crops. *Insects* **2020**, *11*, 536, doi:10.3390/insects11080536.

21. de Souza, L.; Cardoso, M. das G.; Konig, I.F.M.; Ferreira, V.R.F.; Caetano, A.R.S.; Campolina, G.A.; Haddi, K. Toxicity, Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium Verum* Essential Oil in *Drosophila Suzukii*. *Agriculture* **2022**, *12*, 1667, doi:10.3390/agriculture12101667.

22. Finetti, L.; Civolani, S.; Mirandola, D.; Benetti, L.; Francati, S.; Albanese, F.; Menicucci, F.; Michelozzi, M.; Bellardi, M.G.; Dindo, M.L.; et al. Monarda Didyma Hydrolate Affects the Survival and the Behaviour of *Drosophila Suzukii*. *Insects* **2022**, *13*, 280, doi:10.3390/insects13030280.

23. Caetano, A.R.S.; Cardoso, M. das G.; Haddi, K.; Campolina, G.A.; Souza, B.M.; Lunguinho, A.; Souza, L.; Nelson, D.L.; Oliveira, J.E. *Rosmarinus Officinalis* Essential Oil Incorporated into Nanoparticles as an Efficient Insecticide against *Drosophila Suzukii* (Diptera: Drosophilidae). *Austral Entomol.* **2022**, *61*, 265–272, doi:10.1111/aen.12592.

24. Batish, D.R.; Singh, H.P.; Kohli, R.K.; Kaur, S. *Eucalyptus* Essential Oil as a Natural Pesticide. *For. Ecol. Manage.* **2008**, *256*, 2166–2174, doi:10.1016/j.foreco.2008.08.008.

25. Dhakad, A.K.; Pandey, V. V; Beg, S.; Rawat, J.M.; Singh, A. Biological, Medicinal and Toxicological Significance of *Eucalyptus* Leaf Essential Oil: A Review. *J. Sci. Food*

Agric. **2018**, *98*, 833–848, doi:10.1002/jsfa.8600.

26. Salehi, B.; Sharifi-Rad, J.; Quispe, C.; Llaique, H.; Villalobos, M.; Smeriglio, A.; Trombetta, D.; Ezzat, S.M.; Salem, M.A.; Zayed, A.; et al. Insights into *Eucalyptus* Genus Chemical Constituents, Biological Activities and Health-Promoting Effects. *Trends Food Sci. Technol.* **2019**, *91*, 609–624, doi:10.1016/j.tifs.2019.08.003.

27. Andreazza, F.; Bernardi, D.; Marangon, R.B.; Botton, M.; Nava, D.E. Técnica de Criação de *Drosophila Suzukii* (Matsumura, 1931)(Diptera: Drosophilidae) Em Dieta Artificial. *Embrapa Clima Temperado-Boletim Pesqui. e Desenvolv. (INFOTECA-E).* **2016**, 23.

28. Mendonca, L. de P.; Oliveira, E.E.; Andreazza, F.; Rezende, S.M.; Faroni, L.R.D.; Guedes, R.N.C.; Haddi, K. Host Potential and Adaptive Responses of *Drosophila Suzukii* (Diptera: Drosophilidae) to Barbados Cherries. *J. Econ. Entomol.* **2019**, doi:10.1093/jee/toz195.

29. WFN WFN Óleos Essenciais Available online: https://www.wnf.com.br/.

30. Yeom, H.-J.; Kang, J.; Kim, S.-W.; Park, I.-K. Fumigant and Contact Toxicity of Myrtaceae Plant Essential Oils and Blends of Their Constituents against Adults of German Cockroach (*Blattella Germanica*) and Their Acetylcholinesterase Inhibitory Activity. *Pestic. Biochem. Physiol.* **2013**, *107*, 200–206, doi:10.1016/j.pestbp.2013.07.003.

31. Erland, L.A.E.; Rheault, M.R.; Mahmoud, S.S. Insecticidal and Oviposition Deterrent Effects of Essential Oils and Their Constituents against the Invasive Pest *Drosophila Suzukii* (Matsumura) (Diptera: Drosophilidae). *Crop Prot.* **2015**, *78*, 20–26, doi:10.1016/j.cropro.2015.08.013.

32. Jang, M.; Kim, J.; Yoon, K.A.; Lee, S.H.; Park, C.G. Biological Activity of Myrtaceae Plant Essential Oils and Their Major Components against *Drosophila Suzukii* (Diptera: Drosophilidae). *Pest Manag. Sci.* **2017**, *73*, 404–409, doi:10.1002/ps.4430.

33. Kim, J.; Jang, M.; Shin, E.; Kim, J.; Lee, S.H.; Park, C.G. Fumigant and Contact Toxicity of 22 Wooden Essential Oils and Their Major Components against *Drosophila Suzukii* (Diptera: Drosophilidae). *Pestic. Biochem. Physiol.* **2016**, *133*, 35–43, doi:10.1016/j.pestbp.2016.03.007.

34. Bedini, S.; Cosci, F.; Tani, C.; Pierattini, E.C.; Venturi, F.; Lucchi, A.; Ioriatti, C.; Ascrizzi, R.; Flamini, G.; Ferroni, G.; et al. Essential Oils as Post-Harvest Crop Protectants against the Fruit Fly *Drosophila Suzukii*: Bioactivity and Organoleptic Profile. *Insects* **2020**, *11*, 508, doi:10.3390/insects11080508.

35. Fernández-Bedmar, Z.; Anter, J.; de La Cruz-Ares, S.; Muñoz-Serrano, A.; Alonso-Moraga, Á.; Pérez-Guisado, J. Role of Citrus Juices and Distinctive Components in the Modulation of Degenerative Processes: Genotoxicity, Antigenotoxicity, Cytotoxicity, and Longevity in *Drosophila*. *J. Toxicol. Environ. Heal. Part A* **2011**, *74*, 1052–1066, doi:10.1080/15287394.2011.582306.

36. IOANNOU, C.S.; PAPADOPOULOS, N.T.; KOULOUSSIS, N.A.; TANANAKI, C.I.; KATSOYANNOS, B.I. Essential Oils of Citrus Fruit Stimulate Oviposition in the Mediterranean Fruit Fly *Ceratitis Capitata* (Diptera: Tephritidae). *Physiol. Entomol.* **2012**, *37*, 330–339, doi:10.1111/j.1365-3032.2012.00847.x.

37. Silva, S.M.; Haddi, K.; Viteri Jumbo, L.O.; Oliveira, E.E. Progeny of the Maize Weevil, *Sitophilus Zeamais* , Is Affected by Parental Exposure to Clove and *Cinnamon* Essential Oils. *Entomol. Exp. Appl.* **2017**, *163*, 220–228, doi:10.1111/eea.12559.

38. Vayndorf, E.M.; Lee, S.S.; Liu, R.H. Whole Apple Extracts Increase Lifespan, Healthspan and Resistance to Stress in *Caenorhabditis Elegans*. *J. Funct. Foods* **2013**, *5*, 1235–1243, doi:10.1016/j.jff.2013.04.006.

39. Wang, J.; Deng, N.; Wang, H.; Li, T.; Chen, L.; Zheng, B.; Liu, R.H. Effects of Orange Extracts on Longevity, Healthspan, and Stress Resistance in *Caenorhabditis Elegans*. *Molecules* **2020**, *25*, 351, doi:10.3390/molecules25020351.

40. Shimizu, C.; Wakita, Y.; Inoue, T.; Hiramitsu, M.; Okada, M.; Mitani, Y.; Segawa, S.; Tsuchiya, Y.; Nabeshima, T. Effects of Lifelong Intake of Lemon Polyphenols on Aging and Intestinal Microbiome in the Senescence-Accelerated Mouse Prone 1 (SAMP1). *Sci. Rep.* **2019**, *9*, 3671, doi:10.1038/s41598-019-40253-x.

41. Guedes, R.N.C.; Walse, S.S.; Throne, J.E. Sublethal Exposure, Insecticide Resistance, and Community Stress. *Curr. Opin. Insect Sci.* **2017**, *21*, 47–53, doi:10.1016/j.cois.2017.04.010.

42. Guedes, R.N.C.; Cutler, G.C. Insecticide-Induced Hormesis and Arthropod Pest Management. *Pest Manag. Sci.* **2014**, *70*, 690–697, doi:10.1002/ps.3669.

43. Zou, S.; Carey, J.R.; Liedo, P.; Ingram, D.K.; Müller, H.-G.; Wang, J.-L.; Yao, F.; Yu, B.; Zhou, A. The Prolongevity Effect of Resveratrol Depends on Dietary Composition and Calorie Intake in a Tephritid Fruit Fly. *Exp. Gerontol.* **2009**, *44*, 472–476, doi:10.1016/j.exger.2009.02.011.

44. Lee, K.-S.; Lee, B.-S.; Semnani, S.; Avanesian, A.; Um, C.-Y.; Jeon, H.-J.; Seong, K.-M.; Yu, K.; Min, K.-J.; Jafari, M. Curcumin Extends Life Span, Improves Health Span, and Modulates the Expression of Age-Associated Aging Genes in *Drosophila Melanogaster*. *Rejuvenation Res.* **2010**, *13*, 561–570, doi:10.1089/rej.2010.1031.

45. Amiri, A.; Bandani, A.R. Does Timing of Post-Stressor Exposure Mating Matter for Parental Effect? *J. Stored Prod. Res.* **2022**, *99*, 102021, doi:10.1016/j.jspr.2022.102021.

46. Salmon, A.B.; Marx, D.B.; Harshman, L.G. A Cost of Reproduction in *Drosophila Melanogaster*: Stress Susceptibility. *Evolution (N. Y).* **2001**, *55*, 1600–1608, doi:10.1111/j.0014-3820.2001.tb00679.x.

47. Haddi, K.; Mendes, M. V.; Barcellos, M.S.; Lino-Neto, J.; Freitas, H.L.; Guedes, R.N.C.; Oliveira, E.E. Sexual Success after Stress? Imidacloprid-Induced Hormesis in Males of the Neotropical Stink Bug *Euschistus Heros*. *PLoS One* **2016**, *11*, e0156616, doi:10.1371/journal.pone.0156616.

48. Blouquy, L.; Mottet, C.; Olivares, J.; Plantamp, C.; Siegwart, M.; Barrès, B. How Varying Parameters Impact Insecticide Resistance Bioassay: An Example on the Worldwide Invasive Pest *Drosophila Suzukii*. *PLoS One* **2021**, *16*, e0247756, doi:10.1371/journal.pone.0247756.

49. Haddi, K.; Viteri Jumbo, L.O.; Costa, M.S.; Santos, M.F.; Faroni, L.R.A.; Serrão, J.E.; Oliveira, E.E. Changes in the Insecticide Susceptibility and Physiological Trade-Offs Associated with a Host Change in the Bean Weevil *Acanthoscelides Obtectus*. *J. Pest Sci. (2004).* **2018**, *91*, 459–468, doi:10.1007/s10340-017-0860-1.

50. Flatt, T.; Kawecki, T.J. Juvenile Hormone as a Regulator of the Trade-off between Reproduction and Life Span in *Drosophila Melanogaster*. *Evolution (N. Y).* **2007**, *61*,

1980–1991, doi:10.1111/j.1558-5646.2007.00151.x.

51. Jager, T.; Barsi, A.; Ducrot, V. Hormesis on Life-History Traits: Is There Such Thing as a Free Lunch? *Ecotoxicology* **2013**, *22*, 263–270, doi:10.1007/s10646-012-1022-0.

52. Calabrese, E.J.; Kozumbo, W.J. The Hormetic Dose-Response Mechanism: Nrf2 Activation. *Pharmacol. Res.* **2021**, *167*, 105526, doi:10.1016/j.phrs.2021.105526.

53. He, F.; Ru, X.; Wen, T. NRF2, a Transcription Factor for Stress Response and Beyond. *Int. J. Mol. Sci.* **2020**, *21*, 4777, doi:10.3390/ijms21134777.

54. Bottino-Rojas, V.; Talyuli, O.A.C.; Carrara, L.; Martins, A.J.; James, A.A.; Oliveira, P.L.; Paiva-Silva, G.O. The Redox-Sensing Gene Nrf2 Affects Intestinal Homeostasis, Insecticide Resistance, and Zika Virus Susceptibility in the Mosquito *Aedes Aegypti*. *J. Biol. Chem.* **2018**, *293*, 9053–9063, doi:10.1074/jbc.RA117.001589.

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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		

Figures captions

Figure 1. Longevity of 5-7 day old mated females (A) and males (B) of *Drosophila suzukii* exposed to low concentrations (LC_0, CL_5 and CL_{20}) of *Eucaliptus globulus* essential oil.

Figure 2. Total number (A), number of females (B) and males (C) of emerged progeny flies of 5-7 days old mated *Drosophila suzukii* exposed to low concentrations (LC_0, CL_5 and CL_{20}) of *Eucaliptus globulus* essential oil.

Figure 3. Longevity 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 *Eucaliptus globulus* essential oil.

Figure 4. Total number (A), number of females (B) and males (C) of emerged progeny flies of newly emerged virgin *Drosophila suzukii* females (A) and males (B) paired in four couple's 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 *Eucaliptus globulus* essential oil.

Supplementary Figure 1. Body mass of pupae (A), adults flies (B) progeny of *Drosophila suzukii* exposed to low concentrations (LC_0, CL_5 and CL_{20}) of *Eucaliptus 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.

Figure 1:

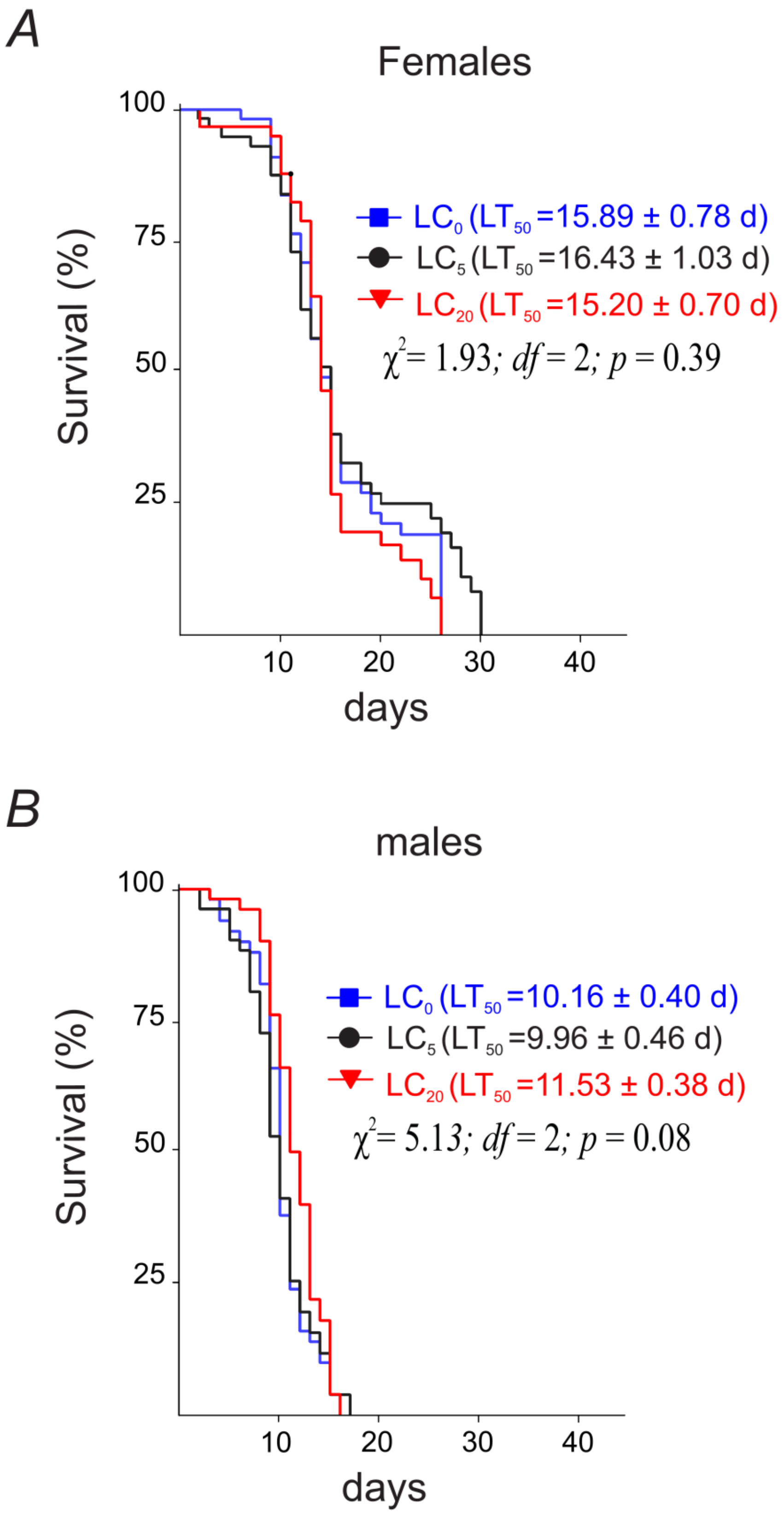

A
Females
100
75
50
25
Survival (%)
10
20
30
40
days
LC$_0$ (LT$_{50}$ =15.89 ± 0.78 d)
LC$_5$ (LT$_{50}$ =16.43 ± 1.03 d)
LC$_{20}$ (LT$_{50}$ =15.20 ± 0.70 d)
χ^2= 1.93; df = 2; p = 0.39
B
males
100
75
50
25
Survival (%)
10
20
30
40
days
LC$_0$ (LT$_{50}$ =10.16 ± 0.40 d)
LC$_5$ (LT$_{50}$ =9.96 ± 0.46 d)
LC$_{20}$ (LT$_{50}$ =11.53 ± 0.38 d)
χ^2= 5.13; df = 2; p = 0.08

Figure 2:

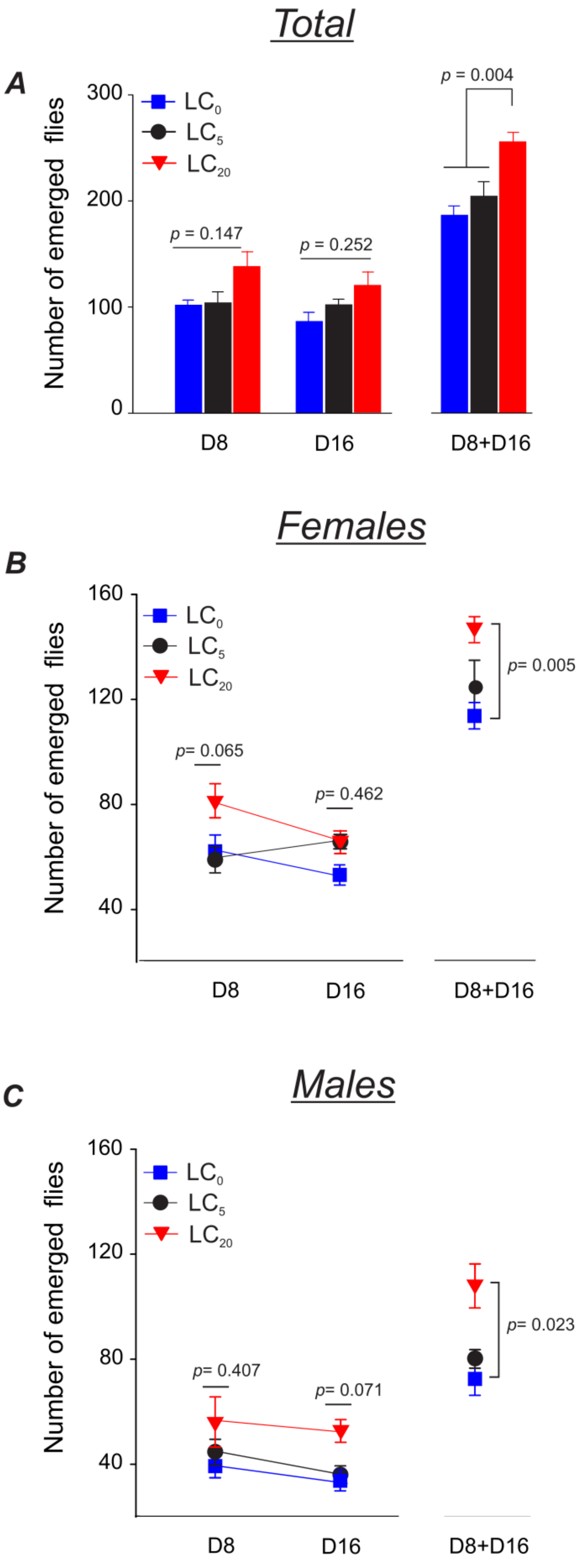

Total
A
Number of emerged flies
300
200
100
0
LC_0
LC_5
LC_{20}
p = 0.147
p = 0.252
p = 0.004
D8
D16
D8+D16
Females
B
Number of emerged flies
160
120
80
40
LC_0
LC_5
LC_{20}
p= 0.065
p= 0.462
p= 0.005
D8
D16
D8+D16
Males
C
Number of emerged flies
160
120
80
40
LC_0
LC_5
LC_{20}
p= 0.407
p= 0.071
p= 0.023
D8
D16
D8+D16

Figure 3:

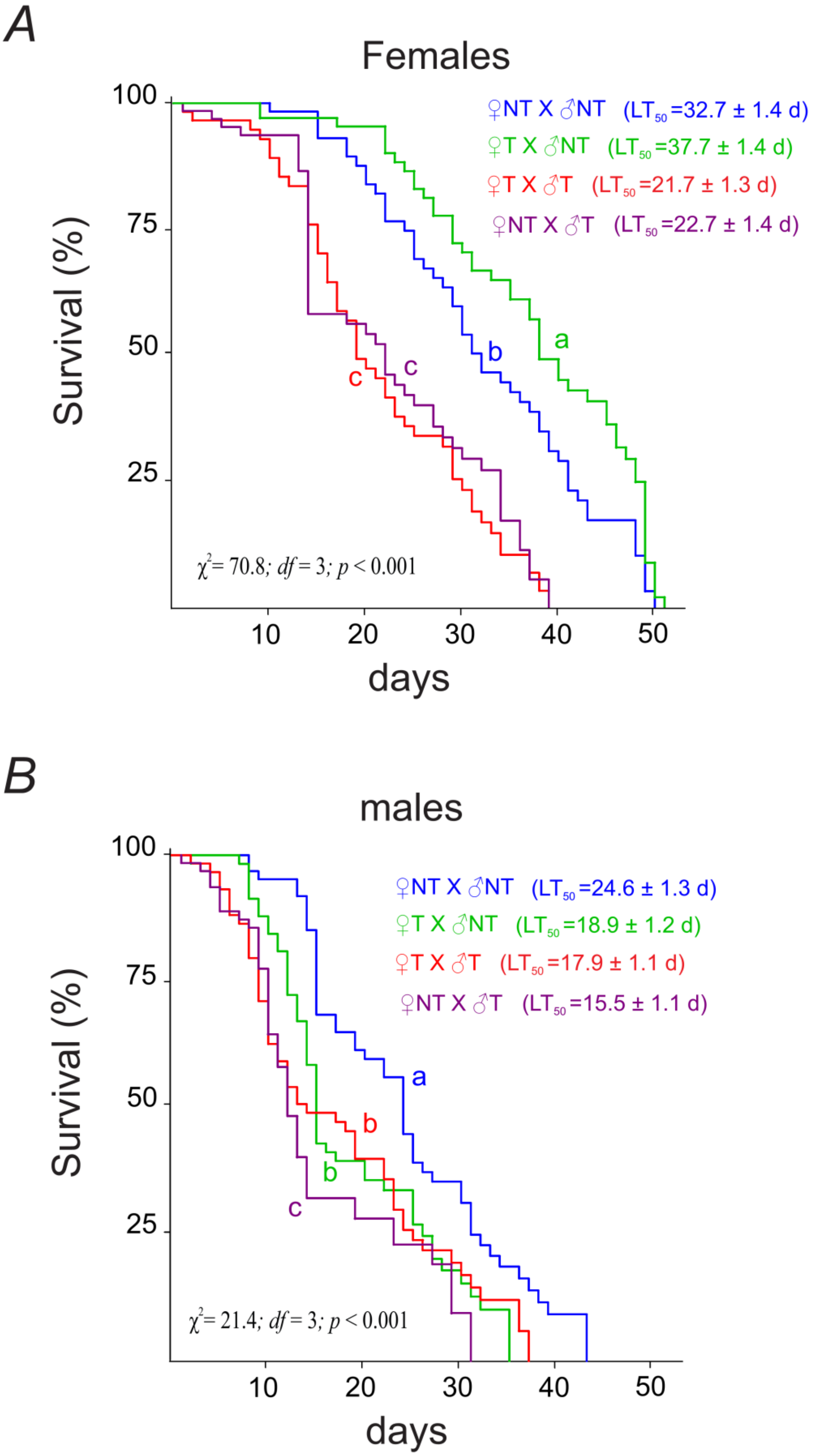
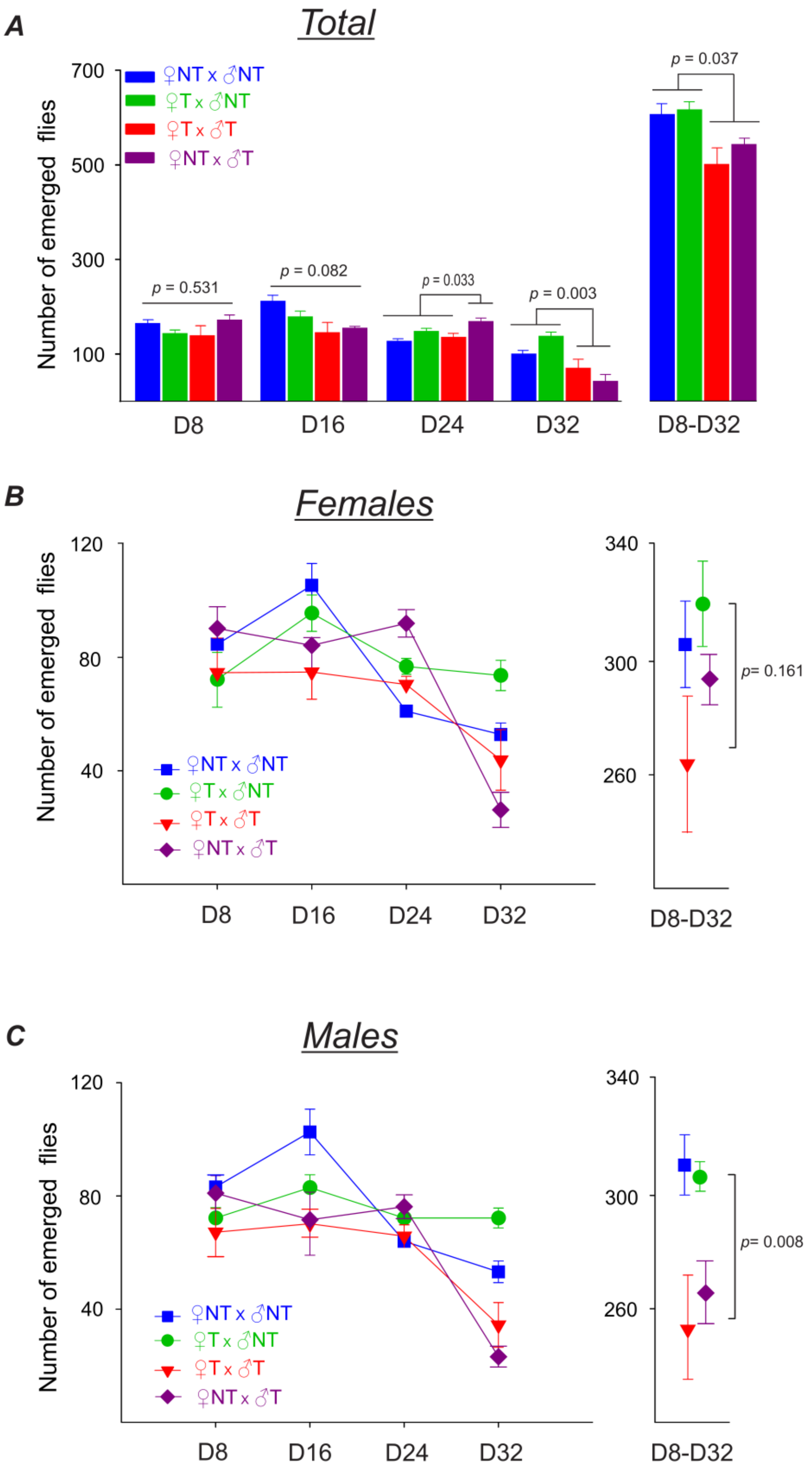
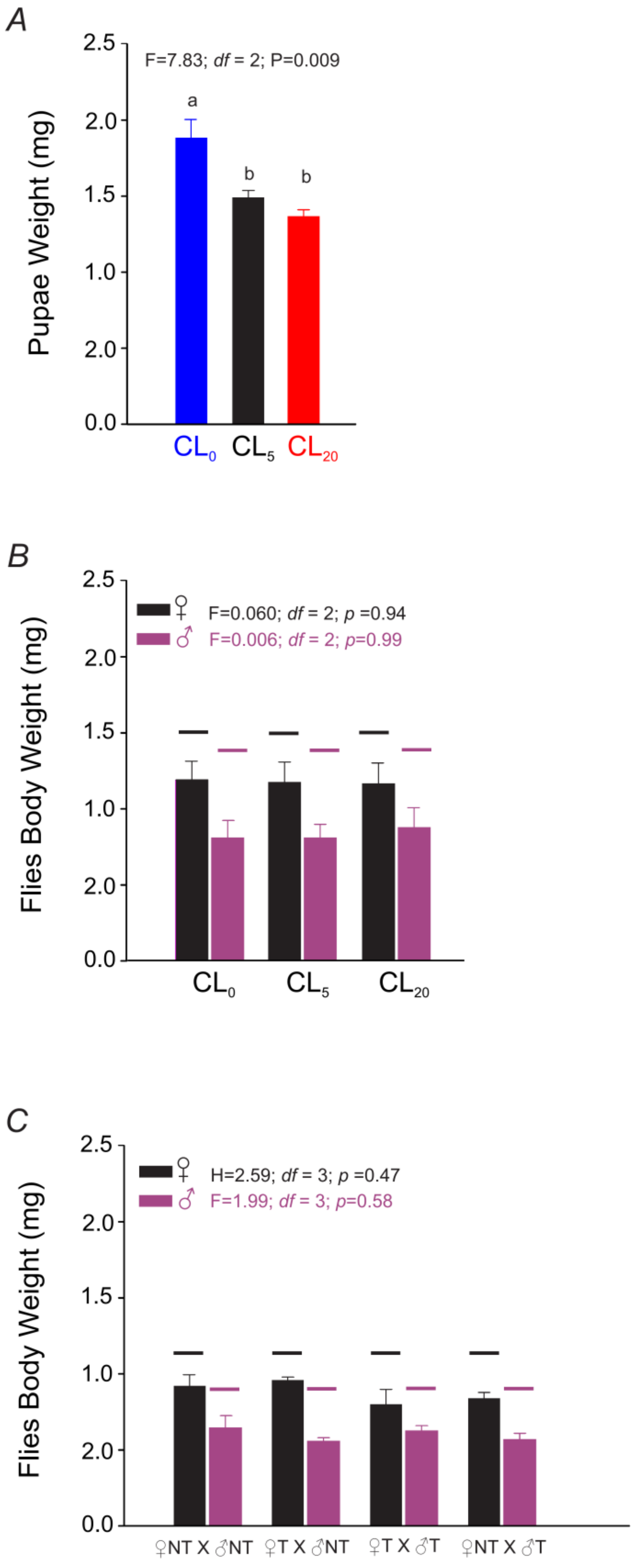

A
Females
Survival (%)
100
75
50
25
10
20
30
40
50
days
♀NT X ♂NT (LT50 =32.7 ± 1.4 d)
♀T X ♂NT (LT50 =37.7 ± 1.4 d)
♀T X ♂T (LT50 =21.7 ± 1.3 d)
♀NT X ♂T (LT50 =22.7 ± 1.4 d)
a
b
c
c
χ2= 70.8; df = 3; p < 0.001
B
males
Survival (%)
100
75
50
25
10
20
30
40
50
days
♀NT X ♂NT (LT50 =24.6 ± 1.3 d)
♀T X ♂NT (LT50 =18.9 ± 1.2 d)
♀T X ♂T (LT50 =17.9 ± 1.1 d)
♀NT X ♂T (LT50 =15.5 ± 1.1 d)
a
b
b
c
χ2= 21.4; df = 3; p < 0.001

Figure 4:


A
Total
Number of emerged flies
700
500
300
100
♀NT x ♂NT
♀T x ♂NT
♀T x ♂T
♀NT x ♂T
p = 0.531
p = 0.082
p = 0.033
p = 0.003
p = 0.037
D8
D16
D24
D32
D8-D32
B
Females
Number of emerged flies
120
80
40
340
300
260
p= 0.161
♀NT x ♂NT
♀T x ♂NT
♀T x ♂T
♀NT x ♂T
D8
D16
D24
D32
D8-D32
C
Males
Number of emerged flies
120
80
40
340
300
260
p= 0.008
♀NT x ♂NT
♀T x ♂NT
♀T x ♂T
♀NT x ♂T
D8
D16
D24
D32
D8-D32

Supplementary figure 1:



agriculture

MDPI

Article

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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Citation: Pineda, M.; Alves, E.L.d.A.; Antunes, J.A.; Carvalho, V.d.C.; Haddi, K. Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. *Agriculture* **2023**, *13*, 404. https://doi.org/10.3390/agriculture13020404

Academic Editor: Yan Wang

Received: 10 January 2023
Revised: 2 February 2023
Accepted: 7 February 2023
Published: 9 February 2023

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

ARTICLE II

Insecticide efficacy and effects of low concentrations of spinosyns on *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) and the parasitoid *Diaschasmimorpha longicaudata* (Hymenoptera: Braconidae)

Entomologia Experimentalis et Applicata Journal standards (preliminary version)

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Abstract

Bioinsecticides of natural origin, such as spinosyns, are widely recommended for fruit fly control throughout the world. Much research is aimed at demonstrating their efficacy in controlling different types of pests, and their high degree of selectivity to non-target insects. However, few studies have been conducted to determine what happens when spinosyn residues are left in the environment and come into direct contact with pests and natural enemies. First, we determined the lethal effect of eight insecticides (organophosphates, pyrethroids, neonicotinoids, spinosyns, pyrroles, and diamides) for the control of *Drosophila suzukii*. Then, we evaluated the sublethal effects (LC_5. $LC_{10,}$ and LC_{20}) of two bioinsecticides (spinetoram and spinosad) in 7-day-old flies adults of *D. suzukii* by assessing the effects on the longevity of the parents, the fertility of the progeny (number of flies emerged weekly) and the weight of pupae and adults. Finally, we determined the lethal (LC_{60}) and sublethal (LC_{10} and LC_{20}) effects on a non-target insect *Diachasmimorpha longicaudata.* Our results demonstrated that commercial insecticides tested Chlorpyrifos, Deltamethrin, Lambda-cyhalothrin; Thiamethoxam, Imidacloprid + Lambda-cyhalothrin; Spinosad, Spinetoram, Chlorfenapyr and Cyantraniprole, have a lethal effect on adults of *D. suzukii.* The spinosyns tested induced a positive effect on longevity, and this was highly correlated with the fertility of the treated flies, evidencing a sex-dependent hormesis effect. Similar effects were observed in *D. longicaudata* where the major hormetic effect was reflected as a change in sex ratio.

Keywords: Bio-insecticide, spinosyns, spotted-wing drosophila, low-concentration, natural enemy.

Introduction

Bioinsecticides are products of natural origin (animal, vegetable, or mineral) that have long been used to control pests (Copping & Menn, 2000; Glare et al., 2012), and continue to be an alternative in the search for new molecules for the development of agrochemicals. Due to their natural origin, bioinsecticides caused a great innovation since they degrade more easily, are more ecological and less harmful to human health, when compared to synthetic insecticides, and have aroused the interest of many farmers, mainly organic food producers, who have included them as an alternative in integrated pest management programs (Copping & Menn, 2000).

Despite the multiple environmental benefits, an important aspect of bioinsecticides to be considered is their structure and physicochemical properties to determine their toxicology (Haddi et al., 2020), as they are composed of natural mixtures of various molecules with different modes of action, and can act as repellents, change their behavior and as growth regulators (Khater, 2012; Guedes et al., 2016). As a result, in recent years, the adverse effects of biorational insecticides have been questioned, especially concerning residues of these products coming into contact with the target species, without causing immediate death, causing other types of long-term consequences, as well as their effects on non-target insects (Desneux et al., 2007; Pavela & Benelli, 2016). Studies of bioinsecticides are focused on the lethal effect on target pests, and most of them are mainly related to their selectivity effect on pollinating insects and natural enemies (Carvalho et al., 2019).

Research on sublethal effects is well documented in multiple investigations showing that prolonged exposure to low concentrations causes changes in biological and behavioral aspects of insects (Stark & Banks, 2003; Guedes et al., 2022). These responses stimulated by low concentrations are called hormetic responses and have been shown to have transgenerational effects (Gutiérres, 2020; Rix et al., 2022). Hormesis is a biphasic dose-response relationship in which a stressor that is toxic at high doses exhibits stimulatory effects at low doses on the pest. Responses caused by hormesis range from changes in developmental timing, to increased fecundity, fertility, longevity, and behavior (Guedes et al., 2022; Cutler et al., 2022). There are many documented studies on the hormetic effect on insects, indicating that, in addition to increasing crop damage, it can contribute to the evolution of resistance and morphological changes (Calabrese., 2008; Brevik et al., 2018; Gibert et al., 2019).

Although there is much information about the hormetic effects caused by conventional insecticides, few part of this research has focused on demonstrating that products of natural origin can induce the same responses (Silva et al., 2017; de Souza et al., 2022; Pineda et al., 2023). Currently, the most widely used bioinsecticides for fruit fly control are the spinosyns. These compounds are the result of the fermentation of a soil microorganism *Saccharopolyspora spinosa* Mertz & Yao (Bacteria: Actinobacteridae) (Mertz & Yao, 1990; Thompson et al., 2000). After multiple investigations and the discovery of the structure of spinosyn A, which mixed with spinosyn D gave rise to spinosad, a broad-spectrum insecticide used to control Lepidoptera, Diptera, and Hemiptera pests. Another benefit of its use was its low toxicity to humans and the environment (Kirst, 2010; Biondi, Mommaerts, et al., 2012; Galm & Sparks, 2016), with the help of other tools such as bioinformatics and genetic engineering, they sought to modify natural spinosyns to create synthetic spinosyns and thus gave rise to spinetoram. A new molecule that is a natural mixture of spinosyns J and L, with more activity-effective, broad-spectrum, and improved residual (Thompson et al., 2000; Sparks et al., 2021). Spinosyns are insecticides that act on the nervous system of insects, interfering with the transmission of nerve signals at gamma-aminobutyric acid (GABA) and acetylcholine receptors, causing hyperexcitation and paralysis resulting in the death of insects (Salgado et al., 2010; Sparks & Nauen, 2015). They are widely used worldwide to control fruit flies.

In fruit crops, the occurrence of fruit fly species and their biological controllers (mainly wasps of the family Braconidae) may vary according to the geographical region. However, it is common to find fruit flies of the genera *Anastrepha*, *Ceratitis*, *Bactrocera* (Diptera: Tephritidae), and *Drosophila* (Diptera: Drosophilidae) (Dias et al., 2018). Within the latter genus, the species *Drosophila suzukii*, known as spotted-wing drosophila, is a pest of Asian origin that was reported in Brazil in 2013, an exotic pest that has polyphagous habits, but has a preference for red fruits such as cherries, strawberries, raspberries, grapes, and blueberries (Deprá et al., 2014). The presence of this pest represents a challenge for fruit growers, as the greatest damage is caused by the larvae that feed on healthy fruits. The management of this pest is focused on the use of traps with toxic baits, cultural practices, and the use of spinosyns, specifically spinetoram, the only insecticide registered to control this pest in Brazil. It should be noted that, despite being very effective in controlling *D. suzukii* and other fruit flies. In addition to not harming the environment, adverse effects on pollinators and natural enemies found in the same environment have already been recorded. Exposure of fruit flies and parasitoids to spinosyn residues has been poorly documented since most research focuses on the selectivity of these products.

Therefore, in this research, we determined the lethal effect of 10 insecticides for the control of *D. suzukii,* in addition to showing that low concentrations of spinosyn induce changes in the longevity and fertility of exposed flies. As well as investigated the exposure of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Parasitoid of *Ceratitis capitata*) to lethal and sublethal doses of spinosyns, where we documented a negative impact on the number of ovipositions and changes in the sex ratio of the progeny.

Material and Methods

Stock colony of *D. suzukii*

The colony of *D. suzukii*. used in the experiments is 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). The flies are maintained in plastic cages (10cm height x 25cm diameter), using artificial diet (for one liter of water: 67 g sugar, 23 g brewer's yeast, 42 g corn flour, 15 g agar, 5.9 ml propionic acid, and 10% nipagin), and following previously described methods (Andreazza et al, 2016; de Souza et al, 2022). The cages are kept under controlled conditions of temperature (T: 23 ± 2^{o}C), relative humidity (RH: 60±5%), and scotophase (12H).

Colony of *Diachasmimorpha longicaudata* and *Ceratitis capitata*

The colony of *D. longicaudata* was established at the Laboratory of Pathology and Microbial Control of Insects, Department of Entomology, Federal University of Lavras, Lavras (Minas Gerais), under natural conditions. The parasitoids were kept in wooden cages (30 x 30 x 30 cm), lined with paper, with the sides covered with voile fabric. The upper face contained a double covering with voile fabric, closed with velcro around the edges, where third instar larvae of *Ceratitis capitata* (Wiedemann 1824) (Diptera: Tephritidae) from the same laboratory were offered daily, reared on an artificial diet adapted from Albajes and Santiago-Álvarez (1980). Inside the cage, water, sugar, and honey were provided as a food source. *C. capitata* flies come from the same laboratory as the parasitoid and are kept in acrylic cages (60 x 31 x 30 cm) containing water, honey, and a mixture of beer yeast and sugar, in a 1:4 ratio. The cage has one side covered with voil cloth, where the female oviposit, just below, it contains plastic containers with water to retain the eggs. Daily, the eggs were collected and deposited in an artificial diet where they remained until the third instar (Albajes & Santiago-Alvarez. 1980).

Insecticides

The following ten commercial formulations of insecticides were assessed: (1) thiamethoxam (Actara® 250WP), (2) cyantraniliprole (Benevia®), (3) chlorpyrifos (Capataz®), (4) spinetoram (Delegate®), (5) deltamethrin (Deltamax 25SC), (6) thiamethoxam + lambda-cyhalothrin (Engeo pleno™ S), (7) imidacloprid (Evidence® 700WG), (8) chlorfenapyr (Pirate), (9) lambda-cyhalothrin (Termimax 25SC), (10) spinosad (Tracer®) (Table 1).

Toxicity and dose-response curves of insecticides on *D. suzukii* adults

Toxicity bioassays were performed as described in the Insecticide Resistance Action Committee (IRAC) protocol No. 26 (IRAC. 2011) with slight modifications (de Souza et al, 2022; Pineda et al, 2023). The experiments were conducted in two stages: first, logarithmically spaced pre-tests of each insecticide were conducted to determine the range of concentrations that cause between 0 and 100% mortality. Second, after determining the range of mortality, 7 to 9 concentrations were selected to determine the dose-response curve for each insecticide. The serial concentrations were prepared by diluting to the final concentration the corresponding insecticide volumes in a 20% sugar water solution. Then, dental cotton rolls (2 cm) were impregnated with 2.2 mL of the prepared serial dilutions of the insecticides and placed in 200 mL glass flasks. As a negative control, a sugar water (20%) solution was used. Subsequently, for each repetition, 20 to 25 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, ensuring normal oxygen flow, and transferred to a BOD with controlled conditions (Temperature: 23±2°C, relative humidity: 60±5%, and scotophase:12H). After 48 hours of exposure, mortality was evaluated with the aid of a soft brush. Flies that did not show any movement were considered dead.

Exposure of *D. suzukii* adults to spinosyns low concentrations

Once the dose-response curve of spinosyns (spinetoram and spinosad) was determined, the concentrations LC_0 (control: sugar water solution 20%), LC_5, $LC_{10,}$ and LC_{20} were selected to assess the effect of low dose exposure on the survival and reproductive output of exposed flies (Supplementary Table 1). The exposure was performed as previously described, and females and males of the same age (5-7 days) were exposed separately to each concentration (PINEDA et al., 2013). After 48 hours, mortality was evaluated and the survivors were transferred to new containers with sugar water solution (20% w/v). After 24 hours, ten pairs from each concentration were formed and transferred to glass containers with 30 mL of artificial diet, with five repetitions for each concentration including the control treatment. The daily mortality of the parents was evaluated, and every 8 days the survivors were transferred to new glass with diet. For each concentration, 10 pupae were taken to estimate their weight. The daily number of emerged flies for each treatment was counted and the body weight of newly emerged (less than 1 day) females (50) and males (50) was also determined.

Exposure to spinosyns on *D. longicaudata*

Based on the dose-response curve performed for *D. suzukii*, as explained above, lethal (LC_{60}) and two sublethal (LC_{10}, LC_{20}) concentrations of spinosade and spinoteram were selected (Supplementary Table 1). Toxicity bioassays on adult wasps were performed as described above. The food substrate containing the corresponding concentration of insecticide was a sugar solution (20%), which was applied (2.2 ml) to a cotton dental roll (2 cm long) placed inside a glass jar, covered with foam, where ten pairs, of 10 females (7 days) and 10 males (5 days) were exposed to each concentration (repetition). The experimental design was completely randomized, with four replicates per treatment and each replicate being composed of a cage containing 10 adult couples of *D. longicaudata*. After 48 hours, mortality was evaluated and the survivors were transferred to a cage consisting of a plastic container (750 mL). Each cage contained a roll of cotton wool with water and a drop of honey as food for the adults. The upper part has holes to allow ventilation and in the center a fine mesh bag (7 x 3.5 cm). Subsequently, 50 third instar larvae of *Ceratitis capitata* were offered as oviposition substratum. After 24 hours the larvae exposure was removed, and after 3 days the oviposition scars per pupa were verified, using a stereomicroscope (Zeiss Stemi 2000-C) (Supplementary Figure 1). The pupae were checked daily for 17 days to count the number of flies or parasitoids that emerged. After that time, pupae without the emergency of parasitoids were dissected, to determine if the parasitoid was non-viable. We consider parasitism successful when there is no fly emergence. If the pupa was empty, it was considered superparasitism, while if the parasitoid larva or adult was black and without movement, it was considered parasitized but non-viable (Supplementary material figure 1). The following parameters were determined: (1) Mortality of adults exposed to lethal and sublethal concentrations of spinosyns; (2) Total number of pupae attacked, with at least one oviposition scar; (3) Emergence percentage, calculated by dividing the number of emerged adults by the total number of larvae and multiplying the result by 100; (4) Parasitism percentage, we consider successful parasitism. When no flies emerged (emerged adult parasitoids. non-viable pupae, super parasitized pupae, and pupae with viable parasitoids that did not emerge), and (5) Sex ratio, as the ratio of males to females emerged.

Statistical analysis

The concentration–mortality data were subjected to probit analysis to estimate lethal (LC_{50} and LC_{95}) and sub-lethal doses ($CL_{5.}$ LC_{10} and LC_{20}) and chi-square (χ^2) values with 95% confidence limits using the SAS V9 statistical software package (SAS Institute, Cary, NC, USA). The insects that remained alive at the end of the bioassay were censored for the analyses. Overall similarity between the survival and median survival times (LT_{50} values) was tested using the χ^2 log-rank test. The results of the survival were subjected to survival analysis were performed using Kaplan-Meier estimators (χ^2 log-rank test) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The total number of flies that emerged, pupae and adult weights, of *D. suzukii*, and the number of live insects of *D. longicaudata*, parasitism rate, and the sex ratio were subjected to univariate Analysis of Variance (one-way ANOVA or two-way 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 ($P \leq 0.05$), Fisher's Least Significance Difference (LSD), or Dunnett's test. Where appropriate, regression analyses were performed to detect trends in the daily emergence of *D. longicaudata* females and males from each treatment over time. The regression model was chosen based on parsimony, low standard errors, and steep increases in R^2 with model complexity. The regression models for each treatment were considered different from each other if the confidence limits of their parameters did not overlap, using the curve fitting procedure of SigmaPlot 12.0.

Results

Lethal toxicity bioassays

The analyses of the concentration-mortality results were based on the low χ^2 values and high P values (p >0.05) obtained using the probit model. All insecticides showed differential toxicities to adults of *D. suzukii* (Table 2). The calculated lethal concentrations (LC_{50}) showed that the most toxic insecticide was Chlorpyrifos (6.10^{-6} mg active ingredient/L) when compared to lambda-cyhalothrin (9.10^{-5} mg/L), thiamethoxam+ lambda-cyhalothrin (3.10^{-4} 2.10^{-4}mg/L), deltamethrin (2.10^{-4}mg/L), spinosad (7.10^{-4} mg/L), chlorfenapyr (1.10^{-2}mg/mL), imidacloprid (0.10^{-2}), cyantraniliprole (2.10^{-3} mg/L), thiamethoxam (3.10^{-2} mg/L) and spinetoram (6.10^{-1} mg/L).

Effects of low concentrations of spinosyns on the biological and reproductive outputs of *D. suzukii* flies

Parental Flies' Longevity

The longevity of *D. suzukii* was significantly affected when were exposed to sublethal concentrations of spinetoram (Log-Rank test, ♀ $\chi^2 = 14.61$, $df = 3$, $P = 0.002$; ♂ $\chi^2 = 15.89$, $df = 3$, $P = 0.001$) (figures 1A-B). When exposed to spinosad. Only the longevity of males showed statistical differences (Log-Rank test, ♀ $\chi^2 = 2.93$, $df = 3$ $P = 0.403$; ♂ $\chi^2 = 64.26$, $df = 2$, $P < 0.001$) (figures 1C-D). The half-life (LT_{50}) of adults exposed to low concentrations of spinetoram increased LC_5 (♀ 21.8 ± 1.1 d; ♂ 18.3 ± 1.0 days) and LC_{10} (♀ 22.8 ± 1.3 d; ♂ 18.0 ± 1.1 days) when compared to the control LC_0 (♀ 19.19 ± 0.8 d; ♂ 15.0 ± 0.8 days). On the other hand, the longevity of males exposed to the same concentrations of spinosad decreased LC_5 (8.5 ± 0.5 days), LC_{10} (12.47 ± 0.6 days), and LC_0 (♂ 15.19 ± 0.8 days).

Weight of progeny pupae and adults

Pupae weight showed differences with spinetoram (F = 14.06; $df = 3$; $P < 0.001$). But not to spinosad (F = 2.06; $df = 3$; $p = 0.14$) (Figure 2A-B). About flies' body mass, the progeny of male flies when parental flies were exposed to low concentrations of spinetoram were significantly lower (♀H = 1.28, *df* = 3, *P* = 0.73; ♂H = 16.07, *df* = 3, *P* = 0.001). When compared to the spinosad (♀H = 2.62, *df* = 3, *P* = 0.45; ♂H = 0.006, *df* = 3, *P* = 1.00) (Figure 2C-D).

Adult fertility

Parental Flies' Fertility was determined by counting the daily number of females and males who emerged and the total (females and males) adults who emerged at 8 and 24 days. As well as the sum of adults emerged during the whole experiment (Day 8 + 16; and Day 8 + 16 + 24; figure 3). When were exposed to spinetoram, the number of flies was significantly higher in the first week (D8 ♀: F=10.464, *df* = 3, *P*<0.001; D8 ♂: F = 8.173, *df* = 3, *P*<0.001) where adults' emergence at LC_{10} stood out from the others (Figure 3B-C). The behavior of the sum of the number of adults that emerged during the whole experiment was similar (♀D8+16: F=7.210, *df* = 3, *P* = 0.002, ♀ D8+16+24: F=3.408, *df* = 3, *P* = 0.036; Figure 3B; ♂ D8+16: F = 13.109, *df* = 3, *P*<0.001; ♂ D8+16+24: F=0.060, *df* = 3, *P* = 0.004; Figure 3C). There were no significant statistical differences in the progeny of females when exposed to spinosad. However, in males, there was a difference in the total number of males who emerged in the 3 weeks (♂D8+16+24: F=3.106; *df* = 3 *P* = 0.048; Figure 3F). When totaling the number of females and males weekly, the resulting progeny of adult flies exposed to spinetoram showed differences in the first weeks (D8: F=11.176, *df* = 3, *P*<0.001; D16: F = 5.015, *df* = 3, *P* = 0.009; Figure 3A) and those that emerged during the whole experiment (D8+16: F = 10.170, *df* = 3, *P*<0.001; D8+16+24: F=4.355, *df* = 3, *P* = 0.016; Figure 3A) contrary to those exposed to spinosad that did not show differences (Figure 3D).

Effects of lethal and sublethal concentrations of spinosyns on the Parasitism of *D. longicaudata*

Mortality of exposed adults

Analysis of variance showed no significant differences in parasitoid mortality when exposed for 48 and 72 hours, to low concentrations of LC_{10}. LC_{20} and LC_{60} of the spinetoram (48h: F = 1.486, *df* = 3, *P* = 0.268; 72h: F=1.038; *df* = 3, *P* = 0.411) and spinosad (48h: H = 4.546, *df* = 3, *P* = 0.208; 72h: H=3.854; *df* = 3, *P* = 0.278) (supplementary material Figure 2).

Number of oviposition scars per pupae

For adults that survived exposure to spinetoram and spinosad, from the previous bioassay, third instar larvae of *C. capitata* (n=824 and n=736) were exposed. The 99 and 96.7% survived to the pupal stage (Supplementary Material Table 2). The number of oviposition scars per pupa was significantly higher when adults were not treated with insecticide. The analysis of variance showed significant statistical differences for the number of scars per pupae in both experiments (spinetoram H = 151.942, *df* = 3, $P < 0.001$; spinosad, H=163.641, *df* = 3, $P < 0.001$). The number of oviposition scars per parasitized pupa ranged from 1 to 29 scars. The highest number of scars was evidenced in the control (9.49 ± 0.19) compared with scars pupae in LC_{20} (spinetoram 5.37 ± 0.15; spinosad 5.73 ± 0.22) (Figure 4).

Total emergency, sex ratio, and parasitism

The analysis of variance showed that the percentage of total emergence of *D. longicaudata* progeny was not different when parents were exposed to Spinetoram (F= 1.387, *df* = 3, $P = 0.283$), but was different when exposed to spinosad (F = 7.128, *df* = 3, $P = 0.003$) (Figura 5A). About the sex ratio, the analysis of variance showed no statistical differences for any of the spinosyns (spinetoram H = 4.211, *df* = 3, $P = 0.240$; spinosad H = 5.839, *df* = 3, $P = 0.120$) (Figura 5B; Supplementary material Table 3). The analysis of variance showed significant statistical differences, only for the percentage of parasitism of progeny when adults of *D. longicaudata* were exposed to LC_{10}. LC_{20} and LC_{60} of spinetoram (spinetoram H = 9.624, *df* = 3; $P = 0.022$, spinosad; H = 1.500, *df* = 3, $P = 0.682$) (Figura 5C). In all cases, one adult parasitoid was obtained from each pupa of *C. capitate*, from parents exposed to LC_{10}. LC_{20} and LC_{60} concentrations, of spinetoram 594 individuals were obtained and 617 adults were obtained from parents exposed to the same concentrations of spinosad (Supplementary material Table 3). Finally, when we analyze the percentage of emerged adults by sex, the results of the Two Way Analysis of Variance indicated a significant effect of sex (spinetoram F = 16.369, *df* = 1, $P < 0.001$; spinosad F = 26.498, *df* = 1, $P < 0.001$) and the sex-concentration interaction for the two insecticides (spinetoram F = 3.809, *df* = 3, $P = 0.019$; spinosad F = 4.531, *df* = 3, $P = 0.009$) (Figura 6A-B). This difference, in relation to sex, was significantly affected when adults were treated with the different concentrations of spinosyns. It can be observed that the sex ratio was normal (0.5) (Fisher, 1930) in the control, but in the other treatments, there was an increase in males (Figure 5B). To observe the daily peak emergence by sex, a three-parameter Gaussian model curve was used (Figure 7 A-D; Table 3). The results of daily emergence did not show significant results. However, the trend of the nonlinear Simple Exponent, 3-parameter regression of cumulative emergence indicates that the proportion of males (Figure 7 G-H; Table 3), was higher in relation to females (Figure 7 E-F; Supplementary material Table 4), for all treatments, when compared to the control.

Discussion

All insecticides tested were efficient for the control of adults of *D. suzukii.* In this research, we determined the LC_{90} lethal effect of ten insecticides belonging to different chemical groups and broad spectrum (diamides, spinosyns, neonicotinoids, pyrethroids, pyrroles, and organophosphates). All were efficient in controlling adults of *D. suzukii* (Hoffmann Schlesener et al ., 2017). The most neurotoxic insecticide was chlorpyrifos, and according to the calculated toxicity radius would be thiamethoxam+lamba-cyhalothrin >lamba-cyhalothrin >deltamethrin >spinosad >chlorfenapyr >imidacloprid >cyantraniprole >thiamethoxam >spinetoram (Table 2). Thus, we demonstrate other insecticides that can be part of the integrated management of *D. suzukii*, which have different modes of action and can be used as alternatives for insecticide rotation, for fruit production, avoiding the development of genetic resistance. However, another important point is the lack of knowledge when the opposite effect occurs: the residual activity of the applied bioinsecticides. Positive effects on the survival and reproduction of various insect pests when subjected to low concentrations of essential oils have been reported previously (Haddi et al., 2020). In some cases, residues remain in the environment between five and ten days after application, and depending on environmental factors it can be shorter or prolonged (Van Timmeren & Isaacs, 2013), and residues of spinosyns can accumulate in the environment and cause *D. suzukii* adults to be exposed to low concentrations (Stark & Banks, 2003; Biondi, Mommaerts et al., 2012).

The current research reports sublethal effects when 7-day-old adult flies of *D. zusukii* were exposed for 48 hours to LC_5, $LC_{10,}$ and LC_{20} of spinosad and spinetoram. Our results demonstrate a positive relationship between adult longevity and progeny emergence. Adult flies exposed to spinetoram showed greater longevity in the females exposed to LC_{20} (Figure 1A), which was reflected in a greater number of progeny (Figure 3A), in contrast to those exposed to spinosad, where adults were less long-lived (Figure 1C-D), and had fewer progeny (Figure 3D). The pupal weight of the progeny of adults exposed to LC_5, $LC_{10,}$ and LC_{20} of spinetoram were lower in relation to spinosad (Figure 2A). In this case, we demonstrated a negative relationship in relation to adult emergence, which can be explained by the fact that the availability of artificial diet was always 30mL, causing intraspecific competition for food for the developing larvae, which was reflected in the lower weight of pupae and adult progeny of adults treated with spinetoram in relation to the control (Figure 2A-C and 3B-C). As for spinosad, there were no differences in the weight of pupae and adult progenies. In this regard, we can infer that intraspecific competition was lower, considering that the weight of adults was higher, but the number of flies that emerged weekly was lower (Figure 2B-D and 3D). The interaction of individuals of the same species may be reflected in the decrease in food resources, which is directly reflected in the survival, weight, and body size of adults (Salmon et al., 2001; Haddi et al., 2016; Brevik et al., 2018; Beck & Blumer, 2021; Lima-Camara et al., 2022). In general, we observed that the hormetic effect, when spotted wing drosophila are exposed to low concentrations of spinosyns, is directly related to sex, and females are more fertile in the first week of exposure. Previous studies, also report different stimulatory responses provoked by exposure to low doses of spinosyns, the survival rate decreased in *Aedes aegypti* (Diptera: Culicidae) (Wang et al, 2022), in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) the development time increased and reduced larval body weight (Gao et al., 2021), for *Tetranychus urticae* (Acari: Tetranychidae) there was also a decrease in the life cycle (Wang et al., 2016).

Our results show a positive effect on survival and the number of progeny of *D. suzukii*. Different toxicology studies report on the stimulatory response of insects exposed to low doses of insecticides (Guedes & Cutler, 2014; Guedes et al., 2017). These stress responses generated by exposure to low doses of spinosyns may be related to the immune, adaptive, morphological, and antioxidative responses of exposed insects (Rossnerova et al, 2020). In humans, generalized mechanisms based on the redox-activated transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) have already been suggested for hormesis (Calabrese & Kozumbo, 2021). Resposts stimulatory effect through sex-dependent hormetic effects on *D. Suzuki*, with females showing higher survival than males when exposed to low concentrations of spinetoram, and reduced survival when exposed to spinosad. A difference in pupal weight was notable, as it was higher in spinosad compared to spinetoram (Deans et al., 2022). Interestingly, there was no effect on the weight of adults. Negative effects on the growth and development of several insect pests have been reported as adverse effects of sublethal exposure (Teixeira et al., 2009; Lai & Su, 2011). It has been reported that lepidopteran larvae exposed to low concentrations of insecticides can alter physiological processes, causing abnormal metabolism, as a response insects present a nutritional imbalance, which can result in reduced body mass, malformation of pupae and adults (Tanani et al., 2012; Bednarska & Stachowicz, 2013; Mojarab-Mahboubkar, Sendi & Aliakbar, 2015). Importantly, these sublethal effects can carry over to the adult stage, which can lead to tolerance in the offspring of surviving adults through transgenerational hormetic effects (Shahout et al., 2011). Several researchers have put forward several hypotheses for the responses caused by insecticide exposure stress, some related to genetic mutation rates, epigenetic effects, and transcription factors that regulate cellular defense against toxic and oxidative stress (Brevik et al., 2018; Calabrese & Kozumbo, 2021; Pineda et al., 2023). In any case, these mechanisms need to be further studied to better understand and validate insecticide-induced stimulatory responses, especially in pests that are difficult to control such as *D. suzukii*.

Lethal and sub-lethal concentrations of spinosyns can also alter the biology and behavior of biological controllers. *D. longicaudata* is the most important and efficient parasitoid species controlling tephritid pests in Central and South America (Sivinski et al., 1996; Harbi et al., 2018; Suárez et al., 2019). Several investigations are focused on determining the lethal effect of insecticides and their selectivity on natural enemies. Some, such show that spinosyns, organophosphates, pyrethroids, and neonicotinoids are lethal to this parasitoid (Cardoso et al., 2021). In contrast, our results showed no significant differences when adults of the same age, were exposed for 48 and 72 hours to LC_{10}, $LC_{20,}$ and LC_{60} concentrations of spinetoram and spinosad (Supplementary Material Figure 1). This difference may be related to the method of exposure (Desneux et al., 2007; Biondi, Desneux, et al., 2012; Stupp et al., 2020). Similarly, exposure of *D. longicaudata* to toxic baits with Spinetoram and spinosad (LC 0.096g a.i.L^{-1} or kg) does not also cause significant mortality (<10% mortality) (Bernardi et al., 2019). In contrast, studies show that the longer the exposure time of parasitoids to biopesticides, the higher their mortality is greater than 80% and the greater the sublethal effects (Ruiz et al., 2008; Biondi et al., 2013). Although the mortality of *D. longicaudata* adults exposed to spinosad was not significant, the oviposition capacity of females was affected. The number of oviposition scars was significantly higher when fed with water and honey. This effect is directly related if we take into account the impact on the nervous system, and causing involuntary muscle contractions, tremors, and paralysis in treated insects (Salgado, 1998). Although the females survived, we found that their foraging and oviposition capacity was affected 72 hours after ingestion of the bioinsecticide. The decrease in the number of scars and oviposition attempts of treated insects is clearly a physiological and behavioral response caused by spinetoram and spinosad (Umoru et al., 1996; Montoya et al., 2013). In this case, we found that lethal and sublethal doses, of bioinsecticides reduced the number of ovipositor insertions (scars) in the pupae of *C. capitata* (Desneux et al., 2004; Altafini et al., 2013; Rodríguez-Mota et al., 2015). Our results showed that the percentage of parasitism of *D. longicaudata* was not affected by exposure to spinosyns, as it was higher than 95%. A few individuals of *C. capitata* were obtained. However, the percentage of adult parasitoid emergence was lower than expected in spinetoram progeny than in spinosad. In the specific case of the progeny exposed to spinetoram, we verified a higher percentage of non-viable pupae (6.5%), superparasitized pupae (4.40%), and non-parasitized pupae (0.84%) (Supplementary Material Table 3). Our results coincide with several studies that show that the contact of bioinsecticides reduces progeny and has other secondary effects. However, the parasitism rate is not affected, found no reduction in parasitism (Cardoso et al., 2021). The sex ratio differences or longevity of adults exposed to sublethal doses of *D. longicaudata* from commercial formulations of mancozeb, Bordeaux mixture, lime sulfur, and azadirachtin. In relation to daily and cumulative parasitoid emergence by sex, there was a noticeable increase in males in all offspring from parents treated with the spinosyns (Figure 6A-B). Unlike the control where the sex ratio was normal (1:1) (supplementary material Figure 2B). The normal sex ratio in *D. longicaudata* is 0.5 (Fisher,1930). However, the sex ratio can be affected by different external factors such as host quality, superparasitism, and host size (González et al., 2007, 2010; Eizaguirre et al., 2009). The increased production of female offspring in parasitoids is a known arrhenotokous. Where unfertilized eggs become males, fertilized eggs become females (Godfray & J., 1994). A condition that is beneficial for pest control in the field (Heimpel & Lundgren, 2000). However, when there are external disturbances this can vary, the results demonstrate a negative impact on sex ratio when females were exposed to sublethal concentrations of spinosyns, and that change was most significant for offspring of insects exposed to spinosad LC_{60}. We attribute this change as an effect of insecticide contact since all treatments were under equal conditions and the control is within the normal range of sex ratio (1:1). Thus, most studies are focused on lethal effects and selectivity testing of biopesticides, leaving an important bias in the study of the sublethal effect of biopesticides on natural enemies. Other research found that males of *D. longicaudata* were more susceptible to spinosad than females, and reported differential findings in body mass, behavior, and density of sensilla, but did not analyze the sex ratio, reproductive parameters of *D. longicaudata* (Andreazza et al., 2020). It has been proven that insects exposed to insecticides undergo physiological and morphological changes (Gergs et al., 2015). Few studies, for example, Zappalà et al. (2009), reported that in *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) the sex ratio was altered when females were treated with mineral oil. Exposure to sub-lethal concentrations of sphenvalerate on *Chironomus riparius* (Diptera: Chironomidae), resulted in oxidative damage, affected developmental rates, and increased male-to-female ratios (Rodrigues et al., 2015). The fact that there is an increase of males, caused by a negative impact of sublethal doses of spinosyns, will drastically decrease the possibility of mating in this species, directly affecting the natural biological control exerted by this species, considering that unfertilized females will produce male offspring.

Conclusions

All the synthetic insecticides tested are efficient for the control of adult *D. suzukii*, but organophosphates and pyrethroids stood out as being more toxic. We were able to demonstrate that there are positive sex-related effects when spotted wing drosophila adults are exposed to low concentrations of spinosyns, which elicits a hormesis response that directly affects their reproduction. On the other hand, lethal exposure to spinosyns (LC_{60}), for the parasitoid *D. longicaudata* proved to be selective. Nevertheless, when the parasitoid was exposed to low concentrations (LC_{10}; LC_{20}) they evidenced an alteration in the sex ratio of the progeny, showing an increase of males in *D. longicaudata*. Thus, we demonstrate that bioinsecticides also have a negative impact on non-target insects.

Author Contributions M.P.: methodology. investigation. formal analysis. writing—original draft; 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.

Funding: This research was funded by the National Council of Scientific and Technological Development (CNPq). Minas Gerais State Foundation for Research Aid (FAPEMIG. APQ-02230-21), and Coordination for the Improvement of Higher Education Personnel

(Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Capes. Brazil) (Finance code 001).

Institutional Review Board Statement: Not applicable.

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.

References

Agrofit (2023) AGROFIT.

Albajes R & Santiago-Alvarez C (1980) Effects of larval density and of food on the sex ratio of *Ceratitis capitata* (Diptera; Trypetidae). Anales del Instituto Nacional de Investigaciones Agrarias 13:175–182.

Altafini DL. Redaelli LR & Jahnke SM (2013) Superparasitism of *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Florida Entomologist 96:391–395.

Andreason SA. Prabhaker N. Castle SJ. Ganjisaffar F. Haviland DR. Stone-Smith B & Perring TM (2018) Reduced Susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to Commonly Applied Insecticides. Journal of Economic Entomology 111:2340–2348.

Andreazza F. Bernardi D. Marangon RB. Botton M & Nava DE (2016) Técnica de criação de *Drosophila suzukii* (Matsumura. 1931)(Diptera: Drosophilidae) em dieta artificial. Embrapa Clima Temperado-Boletim de Pesquisa e Desenvolvimento (INFOTECA-E).:23.

Andreazza F. Haddi K. Nörnberg SD. Guedes RNC. Nava DE & Oliveira EE (2020) Sex-dependent locomotion and physiological responses shape the insecticidal susceptibility of parasitoid wasps. Environmental Pollution 264:114605.

Beck CW & Blumer LS (2021) Advancing Undergraduate Laboratory Education Using Non-Model Insect Species. Annual Review of Entomology 66:485–504.

Bednarska AJ & Stachowicz I (2013) Costs of living in metal polluted areas: respiration rate of the ground beetle *Pterostichus oblongopunctatus* from two gradients of metal pollution. Ecotoxicology 22:118–124.

Bernardi D. Nondillo A. Baronio CA. Bortoli LC. Junior RM. Treptow RCB. Geisler FCS. Neitzke CG. Nava DE & Botton M (2019) Side effects of toxic bait formulations on *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Scientific Reports 9:12550.

Biondi A. Desneux N. Siscaro G & Zappalà L (2012) Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87:803–812.

Biondi A. Mommaerts V. Smagghe G. Viñuela E. Zappalà L & Desneux N (2012) The non-target impact of spinosyns on beneficial arthropods. Pest Management Science 68:1523–1536.

Biondi A. Zappalà L. Stark JD & Desneux N (2013) Do Biopesticides Affect the Demographic Traits of a Parasitoid Wasp and Its Biocontrol Services through Sublethal Effects? (CJ Salice. Ed. by). PLoS ONE 8:e76548.

Brevik K. Lindström L. McKay SD & Chen YH (2018) Transgenerational effects of insecticides — implications for rapid pest evolution in agroecosystems. Current Opinion in Insect Science 26:34–40.

Bruck DJ. Bolda M. Tanigoshi L. Klick J. Kleiber J. DeFrancesco J. Gerdeman B & Spitler H (2011) Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Management Science 67:1375–1385.

Calabrese EJ (2008) Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry 27:1451.

Calabrese EJ & Kozumbo WJ (2021) The hormetic dose-response mechanism: Nrf2 activation. Pharmacological Research 167:105526.

Cardoso TDN. Stupp P. Rakes M. Martins MB. da Silva Filho JG. Grützmacher AD. Nava DE. Bernardi D & Botton M (2021) Lethal and Sublethal Toxicity of Pesticides Used in Fruit Growing on the Parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae): Implications for Integrated Fruit Fly Management (J Stark. Ed. by). Journal of Economic Entomology 114:2412–2420.

Carvalho GA. Grützmacher AD. Passos LC & de Oliveira RL (2019) Physiological and Ecological Selectivity of Pesticides for Natural Enemies of Insects. Natural Enemies of Insect Pests in Neotropical Agroecosystems. Springer International Publishing. Cham. pp 469–478.

Copping LG & Menn JJ (2000) Biopesticides: a review of their action. applications and efficacy. Pest Management Science 56:651–676.

Cutler GC. Amichot M. Benelli G. Guedes RNC. Qu Y. Rix RR. Ullah F & Desneux N (2022) Hormesis and insects: Effects and interactions in agroecosystems. Science of The Total

Environment 825:153899.

Deprá M. Poppe JL. Schmitz HJ. De Toni DC & Valente VLS (2014) The first records of the invasive pest *Drosophila suzukii* in the South American continent. Journal of Pest Science 87:379–383.

Desneux N. Decourtye A & Delpuech J-M (2007) The Sublethal Effects of Pesticides on Beneficial Arthropods. Annual Review of Entomology 52:81–106.

Desneux N. Pham-Delègue M-H & Kaiser L (2004) Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp. *Aphidius ervi* . Pest Management Science 60:381–389.

Dias NP. Zotti MJ. Montoya P. Carvalho IR & Nava DE (2018) Fruit fly management research: A systematic review of monitoring and control tactics in the world. Crop Protection 112:187–200.

Eizaguirre M. López C. Sans A. Bosch D & Albajes R (2009) Response of *Mythimna unipuncta* Males to Components of the *Sesamia nonagrioides* Pheromone. Journal of Chemical Ecology 35:779–784.

Fisher RA (1930) The genetical theory of natural selection. Oxford University Press. Oxford.

Galm U & Sparks TC (2016) Natural product derived insecticides: discovery and development of spinetoram. Journal of Industrial Microbiology and Biotechnology 43:185–193.

Gao Z. Chen Y. He K. Guo J & Wang Z (2021) Sublethal Effects of the Microbial-Derived Insecticide spinetoram on the Growth and Fecundity of the Fall Armyworm (Lepidoptera: Noctuidae) (T-X Liu. Ed. by). Journal of Economic Entomology 114:1582–1587.

Georghiou GP. Lagunes A & Baker JD (1983) Effect of insecticide rotations on evolution of resistance. Mode of Action. Metabolism and Toxicology. Elsevier. pp 183–189.

Gergs A. Kulkarni D & Preuss TG (2015) Body size-dependent toxicokinetics and toxicodynamics could explain intra- and interspecies variability in sensitivity. Environmental Pollution 206:449–455.

Gibert P. Debat V & Ghalambor CK (2019) Phenotypic plasticity. global change. and the speed of adaptive evolution. Current Opinion in Insect Science 35:34–40.

Glare T. Caradus J. Gelernter W. Jackson T. Keyhani N. Köhl J. Marrone P. Morin L & Stewart A (2012) Have biopesticides come of age? Trends in Biotechnology 30:250–258.

Godfray H & J. C (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press. United Kingdom.

González PI. Montoya P. Perez-Lachaud G. Cancino J & Liedo P (2007) Superparasitism in mass reared *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). a parasitoid of fruit flies (Diptera: Tephritidae). Biological Control 40:320–326.

González PI. Montoya P. Pérez-Lachaud G. Cancino J & Liedo P (2010) Host discrimination and superparasitism in wild and mass-reared *Diachasmimorpha longicaudata* (Hym.: Braconidae) females. Biocontrol Science and Technology 20:137–148.

Gress BE & Zalom FG (2019) Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. Pest Management Science 75:1270–1276.

Guedes RNC & Cutler GC (2014) Insecticide-induced hormesis and arthropod pest management. Pest Management Science 70:690–697.

Guedes RNC. Rix RR & Cutler GC (2022) Pesticide-induced hormesis in arthropods: Towards biological systems. Current Opinion in Toxicology 29:43–50.

Guedes RNC. Smagghe G. Stark JD & Desneux N (2016) Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. Annual Review of Entomology 61:43–62.

Guedes RNC. Walse SS & Throne JE (2017) Sublethal exposure. insecticide resistance. and community stress. Current Opinion in Insect Science 21:47–53.

Gutiérrez Y (2020) Multiple mechanisms in which agricultural insects respond to environmental stressors: canalization. plasticity and evolution. Revista de Ciencias Agrícolas 37:90–99.

Haddi K. Mendes M V.. Barcellos MS. Lino-Neto J. Freitas HL. Guedes RNC & Oliveira EE (2016) Sexual Success after Stress? Imidacloprid-Induced Hormesis in Males of the Neotropical Stink Bug *Euschistus heros* (N Desneux. Ed. by). PLOS ONE 11:e0156616.

Haddi K. Turchen LM. Viteri Jumbo LO. Guedes RN. Pereira EJ. Aguiar RW & Oliveira EE

(2020) Rethinking biorational insecticides for pest management: unintended effects and consequences. Pest Management Science 76:2286–2293.

Harbi A. Sabater-Muñoz B. Beitia F & Chermiti B (2018) Biological control of *Ceratitis capitata* (Diptera: Tephritidae) in Tunisia: introduction and potential establishment of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in Tunisian citrus orchards. IOBC-WPRS Bull. pp 142–151.

Heimpel GE & Lundgren JG (2000) Sex Ratios of Commercially Reared Biological Control Agents. Biological Control 19:77–93.

Hoffmann Schlesener DC. Wollmann J. De Bastos Pazini J. Grützmacher AD & Mello Garcia FR (2017) Effects of insecticides on adults and eggs of *Drosophila suzukii* (Diptera. Drosophilidae). Revista Colombiana de Entomología 43:208.

IRAC (2011) IRAC Susceptibility Test Methods Series. 1:1–2.

Khater HF (2012) Prospects of Botanical Biopesticides in Insect Pest Management. Pharmacologia 3:641–656.

Kirst HA (2010) The spinosyn family of insecticides: realizing the potential of natural products research. The Journal of Antibiotics 63:101–111.

Krüger AP. Scheunemann T. Padilha AC. Pazini JB. Bernardi D. Grützmacher AD. Nava DE & Garcia FRM (2021) Insecticide-mediated effects on mating success and reproductive output of *Drosophila suzukii*. Ecotoxicology 30:828–835.

Lai T & Su J (2011) Effects of chlorantraniliprole on development and reproduction of beet armyworm. *Spodoptera exigua* (Hübner). Journal of Pest Science 84:381–386.

Lima-Camara TN. Medeiros-Sousa AR. Coelho RR & Marrelli MT (2022) Body size does not affect locomotor activity of *Aedes aegypti* and *Aedes albopictus* females (Diptera: Culicidae). Acta Tropica 231:106430.

Mertz FP & Yao RC (1990) Saccharopolyspora spinosa sp. nov. Isolated from Soil Collected in a Sugar Mill Rum Still. International Journal of Systematic Bacteriology 40:34–39.

Mojarab-Mahboubkar. M.. Sendi JJ & Aliakbar A (2015) Effect of *Artemisia annua* L. essential oil on toxicity. enzyme activities. and energy reserves of cotton bollworm *Helicoverpa* *armigera* (Hübner)(Lepidoptera: Noctuidae). Journal of Plant Protection Research 55:4.

Montoya P. Ruiz L. Pérez-Lachaud G. Cancino J & Liedo P (2013) Field superparasitism by *Diachasmimorpha longicaudata* attacking *Anastrepha* spp. larvae on mango fruits. Biological Control 64:160–165.

Pavela R & Benelli G (2016) Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. Trends in Plant Science 21:1000–1007.

Pineda M. Alves EL de A. Antunes JA. Carvalho V de C & Haddi K (2023) Low Concentrations of Eucalyptus Essential Oil Induce Age. Sex. and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. Agriculture 13:404.

Rix RR. Guedes RNC & Christopher Cutler G (2022) Hormesis dose–response contaminant-induced hormesis in animals. Current Opinion in Toxicology 30:100336.

Rodrigues ACM. Gravato C. Quintaneiro C. Barata C. Soares AMVM & Pestana JLT (2015) Sub-lethal toxicity of environmentally relevant concentrations of esfenvalerate to *Chironomus riparius*. Environmental Pollution 207:273–279.

Rodríguez-Mota AJ. Ruíz-Cancino E. Ivanovich-Khalaim A. Coronado-Blanco JM & Treviño-Carreón J (2015) Diversidad de Ichneumonidae (Hymenoptera) en un bosque de Pinus spp. y Juniperus flaccida en Jaumave. Tamaulipas. México. Revista Mexicana de Biodiversidad.

Rossnerova A. Izzotti A. Pulliero A. Bast A. Rattan SIS & Rossner P (2020) The Molecular Mechanisms of Adaptive Response Related to Environmental Stress. International Journal of Molecular Sciences 21:7053.

Ruiz L. Flores S. Cancino J. Arredondo J. Valle J. Díaz-Fleischer F & Williams T (2008) Lethal and sublethal effects of spinosad-based GF-120 bait on the tephritid parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Biological Control 44:296–304.

Salgado VL (1998) Studies on the Mode of action of spinosad: Insect Symptoms and Physiological Correlates. Pesticide Biochemistry and Physiology 60:91–102.

Salgado VL. Sparks TC. Gilbert LI & Gill SS (2010) The spinosyns: chemistry. biochemistry. mode of action. and resistance. Insect Control: Biological and Synthetic Agents. (ed by

SSG Lawrence I. Gilbert) Elsevier Science. pp 207–243.

Salmon AB. Marx DB & Harshman LG (2001) A cost of reproduction in *Drosophila melanogaster*: stress susceptibility. Evolution 55:1600–1608.

Shahout HA. Xu JX. Qiao J. Yao XM & Jia QD (2011) Sublethal Effects of Methoxyfenozide on Growth and Development of Common Cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Research Journal of Biological Sciences 6:58–64.

Silva SM. Haddi K. Viteri Jumbo LO & Oliveira EE (2017) Progeny of the maize weevil. *Sitophilus zeamais*. is affected by parental exposure to clove and *cinnamon* essential oils. Entomologia Experimentalis et Applicata 163:220–228.

Sivinski JM. Calkins CO. Baranowski R. Harris D. Brambila J. Diaz J. Burns RE. Holler T & Dodson G (1996) Suppression of a Caribbean Fruit Fly (*Anastrepha suspensa* (Loew) Diptera: Tephritidae) Population through Augmented Releases of the Parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biological Control 6:177–185.

de Souza L. Cardoso M das G. Konig IFM. Ferreira VRF. Caetano ARS. Campolina GA & Haddi K (2022) Toxicity. Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium verum* Essential Oil in *Drosophila suzukii*. Agriculture 12:1667.

Sparks TC. Crouse GD. Benko Z. Demeter D. Giampietro NC. Lambert W & Brown A V (2021) The spinosyns. spinosad, spinetoram, and synthetic spinosyn mimics - discovery. exploration. and evolution of a natural product chemistry and the impact of computational tools. Pest Management Science 77:3637–3649.

Sparks TC & Nauen R (2015) IRAC: Mode of action classification and insecticide resistance management. Pesticide Biochemistry and Physiology.

Stark JD & Banks JE (2003) Population-level effects of pesticides and other toxicants on arthropods. Annual Review of Entomology 48:505–519.

Stupp P. Rakes M. Martins LN. Piovesan B. da Costa Oliveira D. Contreras Miranda JA. do Prado Ribeiro L. Nava DE & Bernardi D (2020) Lethal and sublethal toxicities of acetogenin-based bioinsecticides on *Ceratitis capitata* and the parasitoid *Diachasmimorpha longicaudata*. Phytoparasitica 48:477–489.

Suárez L. Biancheri MJB. Sánchez G. Murúa F. Funes CF. Kirschbaum DS. Molina D. Laría O & Ovruski SM (2019) Effects of releasing two *Diachasmimorpha longicaudata* population lines for the control of *Ceratitis capitata* infesting three key host fruit species. Biological Control 133:58–65.

Tait G. Mermer S. Stockton D. Lee J. Avosani S. Abrieux A. Anfora G. Beers E. Biondi A. Burrack H. Cha D. Chiu JC. Choi M-Y. Cloonan K. Crava CM. Daane KM. Dalton DT. Diepenbrock L. Fanning P. Ganjisaffar F. Gómez MI. Gut L. Grassi A. Hamby K. Hoelmer KA. Ioriatti C. Isaacs R. Klick J. Kraft L. Loeb G. Rossi-Stacconi MV. Nieri R. Pfab F. Puppato S. Rendon D. Renkema J. Rodriguez-Saona C. Rogers M. Sassù F. Schöneberg T. Scott MJ. Seagraves M. Sial A. Van Timmeren S. Wallingford A. Wang X. Yeh DA. Zalom FG & Walton VM (2021) *Drosophila suzukii* (Diptera: Drosophilidae): A Decade of Research Towards a Sustainable Integrated Pest Management Program (M Brewer. Ed. by). Journal of Economic Entomology 114:1950–1974.

Tait G. Park K. Nieri R. Crava MC. Mermer S. Clappa E. Boyer G. Dalton DT. Carlin S. Brewer L. Walton VM. Anfora G & Rossi-Stacconi MV (2020) Reproductive Site Selection: Evidence of an Oviposition Cue in a Highly Adaptive Dipteran. *Drosophila suzukii* (Diptera: Drosophilidae) (L Stelinski. Ed. by). Environmental Entomology 49:355–363.

Tanani MA. Ghoneim KS & Hamadah KS (2012) Comparative Effects of Certain Igrs on the Carbohydrates of Hemolymph and Fat Body of the Desert Locust. *Schistocerca Gregaria* (Orthoptera: Acrididae). Florida Entomologist 95:928–935.

Teixeira LA. Gut LJ. Wise JC & Isaacs R (2009) Lethal and sublethal effects of chlorantraniliprole on three species of *Rhagoletis* fruit flies (Diptera: Tephritidae). Pest Management Science 65:137–143.

Thompson GD. Dutton R & Sparks TC (2000) Spinosad - a case study: an example from a natural products discovery programme. Pest Management Science 56:696–702.

Van Timmeren S & Isaacs R (2013) Control of spotted wing drosophila. *Drosophila suzukii*. by specific insecticides and by conventional and organic crop protection programs. Crop Protection 54:126–133.

Umoru PA. Powell W & Clark SJ (1996) Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. Bulletin of Entomological Research 86:193–201.

Vontas J. Hernández-Crespo P. Margaritopoulos JT. Ortego F. Feng H-T. Mathiopoulos KD & Hsu J-C (2011) Insecticide resistance in Tephritid flies. Pesticide Biochemistry and Physiology 100:199–205.

Wang L. Pang Z. Chen Q. Song Z. Lu Y. Yang M. Huang J. Yu X & Wang X (2022) Sublethal exposure to spinetoram impacts life history traits and dengue virus replication in Aedes aegypti. Insect Science.

Wang L. Zhang Y. Xie W. Wu Q & Wang S (2016) Sublethal effects of spinetoram on the two-spotted spider mite. *Tetranychus urticae* (Acari: Tetranychidae). Pesticide Biochemistry and Physiology 132:102–107.

Table 1. Characteristics and rates of insecticide formulations used in laboratory bioassays.

Active ingredient	Trade name[1]	Formulation	IRAC MoA group	Manufacturer
Chlorpyrifos	Capataz ®	48%EC	Organophosphates (1B)	Ouro fino química S.A.
Deltamethrin	Deltamax 25SC	2.5%WP	Pyrethroids (3A)	Insetimax indústria química eireli
Lamda-cyhalothrin	Termimax 25SC	10.6% LS		Citromax
Thiamethoxam	Actara® 250WP	25%WG	Neonicotinoids (4A)	Syngenta Proteção de Cultivos Ltda
Imidacloprido	Evidence® 700WG	70%WG		Bayer S.A.
Tiametoxam + lamba-cyhalothrin	Engeo pleno™ S	14.1% CS +10.6%SC	Neonicotinoids (4A) + Pyrethroids (3A)	Syngenta Proteção de Cultivos Ltda. -
Spinosad	Tracer®	48%SC	Spynosyns (5)	Dow agrosciences Industrial Ltda.
Spinetoram	Delegate®	255%WG		Dow agrosciences Industrial Ltda.
Chlorfenapyr	Pirate ®	24% SC	Pyrroles (13)	Basf s.a.
Cyantraniprole	Benevia®	10%OD	Diamide (28)	Fmc química do brasil ltda.

[1] Insecticides used in 2022-laboratory bioassays.

Table 1. Toxicity of commercial insecticides to adult flies of *Drosophila suzukii* toxicity ratio

Trade nome	Active ingredient	No. Insects	LC_{50} (95% FI) mg i.a./L	LC_{95} (95% FI) mg i.a./L	TR^{1} (LC_{50})	χ^{2}	*p*
Capataz ®	**Chlorpyrifos**	**927**	**6.10^{-6} ($5x10^{6}$ – $7x10^{6}$)**	**3.10^{-5} ($2x10^{-5}$-$4x10^{-5}$)**	**---**	**0.346**	**8.95**
Deltamax 25SC	Deltamethrin	805	2.10^{-4} ($2x10^{-4}$ – $2x7.10^{-4}$)	7.10^{-4} ($6x10^{-4}$ – $8x10^{-4}$)	26.88	0.126	8.36
Termimax 25SC	Lamba-cyhalothrin	955	9.10^{-5} ($8x10^{-5}$ – $1x10^{-5}$)	3.10^{-5} ($3x10^{-5}$ -$4x10^{-7}$)	0.99	0.41	7.14
Actara® 250WP	Thiamethoxam	1112	3.10^{-2} (2.9 $x10^{-2}$– 3.7 $x10^{-2}$)	2.10^{-1}(1.6 $x10^{-1}$-2 $x10^{-1}$)	3394.72	0.161	13.03
Evidence® 700WG	Imidacloprid	716	1.10^{-2} (1.2 $x10^{-2}$ – 1.4 $x10^{-2}$)	4.10^{-3} (3 $x10^{-3}$-5 $x10^{-3}$)	141.47	2.54	0.78
Engeo pleno^{TM} S	Thiamethoxam +	710	2.10^{-4} ($2x10^{-4}$ – $3x10^{-4}$)	6.10^{-5} ($5x10^{-5}$-7.10^{-5})	2.10^{-5}	0.4	4.04
	Lamba-cyhalothrin		2.10^{-4} ($2x10^{-4}$ – $2.3x10^{-4}$)	5.10^{-4} ($4x10^{-4}$-$5x10^{-4}$)	2.10^{-5}	0.4	4.04
Tracer®	Spinosad	567	7.10^{-4} ($6x10^{-4}$ – $8x10^{-4}$)	2.10^{-2} (2.3 $x10^{-2}$-3 $x10^{-2}$)	79.04	0.00316	12.36
Delegate®	Spinetoram	980	6.10^{-1} ($6x10^{-1}$– 7.10^{-1})	1.78 (1.58-2.01)	70315.6	0.18	11.4
Pirate ®	Chlorfenapyr	859	1.10^{-1} ($1x10^{-1}$-$1.2x10^{-1}$)	3.10^{-2} (2.7 $x10^{-2}$-4.9 $x10^{-2}$)	118.71	0.345	6.69
Benevia®	Cyantraniprole	877	2.10^{-3}($1.9x10^{-3}$- $2.2x10^{-3}$)	7.10^{-4} (6.10^{-4}-8.10^{-4})	210.52	0.0134	9.78

[1] Toxicity resistence= LC_{50} of insecticide/ LC_{50} of Chlorpyrifos.

Supplemental material (Tables)

Table 1.

Sub-lethal concentrations of two spinosyns determined for *Drosophila suzukii* adult flies .

Active ingredient	**No. Insects**	**LC**	**LC mg i.a./L**	**(95% FI) mg i.a./L**	**X^2**	***p***
Spinetoram	980	LC_5	0.252	0.222-0.281	11.40	0.18
		LC_{10}	0.312	0.280-0.344		
		LC_{20}	0.404	0.370-0.440		
		LC_{60}	0.776	0.720-0.836		
Spinosad	567	LC_5	2.10^{-4}	2.10^{-4}-2.10^{-4}	12.36	0.13
		LC_{10}	2.10^{-4}	2.10^{-4}-3.10^{-4}		
		LC_{20}	3.10^{-4}	3.10^{-4}-4.10^{-4}		
		LC_{60}	9.10^{-4}	8.10^{-4}-9.10^{-4}		

Table 2.

Number of third instar larvae of *C. capitata* offered to surviving adults of *D. longicaudata*, after exposure to different concentrations of spinosyns.

Insecticide	Concentration	Number of larvae exposed	Number of pupae	pupation rate (%)	Larval mortality %	Total number emerged parasitoids
Spineteram	LC_0	207	207	100	0	131
	LC_{10}	202	202	100	0	165
	LC_{20}	215	215	100	0	167
	LC_{60}	200	192	96	4	131
	TOTAL	**824**	**816**	**99.0**	1	**594**
Spinosad	LC_0	194	191	98.5	1.5	146
	LC_{10}	165	155	93.9	6.1	137
	LC_{20}	194	189	96.9	3.1	173
	LC_{60}	183	178	97.3	2.7	161
	TOTAL	**736**	**713**	**96.7**	**3.3**	**617**

Table 3.

Lethal (LC$_{60}$) and sublethal (LC$_{10}$, LC$_{20}$) effects on progeny, adult (F$_1$ generation) of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to spinosyns

Insecticide	Concentration	Larval mortality^{a} %	Pupation^{b} %	Total Emergence^{c} %	Inviability^{d} %	Superparsitism^{e} %	Viable without Emergence^{f} %	No parasitoids^{g} %	Sex ratio^{h}
Spinetoram	LC$_0^*$	0.75	99.25 ± 1.35	69.50 ± 3.16	7.25 ± 1.50	6.125 ± 1.01	1.375 ± 0.49	0.125 ± 0.077	0.49 ± 0.04
	LC$_{10}$	0.00	100 ± 0.00	81.59 ± 2.79	4.75 ± 0.625	2.75 ± 1.125	1.75 ± 0.56	0.00 ± 0.00	0.39 ± 0.02
	LC$_{20}$	0.00	100 ± 0.00	77.47 ± 3.87	4.00 ± 0.75	5.50 ± 0.625	0.50 ± 0.37	2.00 ± 0.75	0.30 ± 0.01
	LC$_{60}$	4.00	96 ± 2.00	67.65 ± 6.18	10.25 ± 3.19	3.25 ± 1.19	0.50 ± 0.37	1.25 ± 0.93	0.43 ± 0.04
Spinosad	LC$_0^*$	0.75	99.25 ± 1.35	69.50 ± 3.16	7.25 ± 1.50	6.125 ± 1.01	1.37 ± 0.49	0.125 ± 0.077	0.49 ± 0.04
	LC$_{10}$	6.10	93,9 ± 0.69	88.46 ± 2.45	4.00 ± 0.75	0.50 ± 0.375	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.08
	LC$_{20}$	3.60	97.4 ± 0.85	91.48 ± 3.48	3.00 ± 0.75	0.25 ± 0.189	0.75 ± 0.37	0.00 ± 0.00	0.33 ± 0.08
	LC$_{60}$	2.70	97.3 ± 1.35	90.36 ± 3.82	3.50 ± 1.25	0.25 ± 0.189	0.50 ± 0.37	0.00 ± 0.00	0.22 ± 0.06

a.
* The same control value was taken into account for all analyses and calculations.
a. Percentage of third instar larvae of *Ceratitis capitata* killed.
b. Percentage of *C. capitata* third instar larvae that developed in pupae
c. Percentage of total adults of *D. longicaudata* obtained from pupae of *C. capitata*
d. Total percentage of non-viable parasitoids *D. longicaudata;* considered as developed parasitoids, but dead inside the host pupa.
e. Consider superparasitism, when after dissection the pupa was totally empty.
f. At the time of dissection, the parasitoid was alive, but its immature state did not allow the sex to be identified.
g. Pupa not parasitized, in this case, an adult of *C. capitata* was obtained.
h. Sex ratio, calculated as follows: Number of females over the total number of emerged insects.

There is a photographic plate to supplement the table 3.

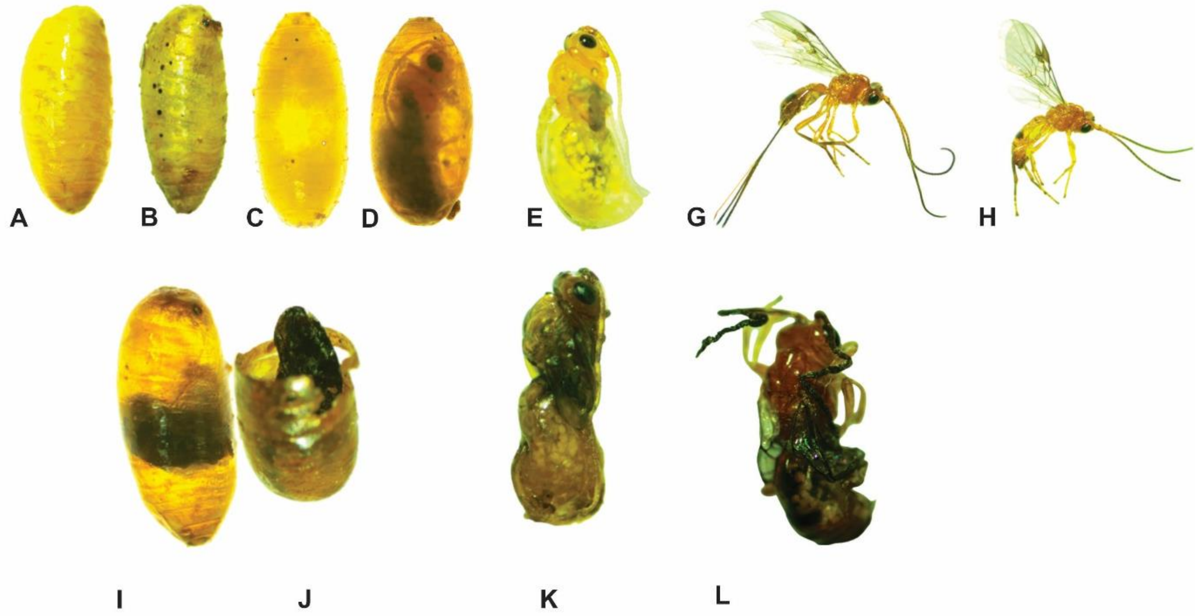

Figure 1. **A**. Pupa normal of *C. capitata*. **B**. parasitized pupa with scars. **C-D**. normal development of *Diachasmimorpha longicaudata*. **E.** Pupa *D. longicaudata*. **G.** Femea **H.** Male *D. longicaudata*. **I-J.** Unviable larvae *D. longicaudata*. **K**. Unviable pupa *D. longicaudata*. **H.** Unviable adult. Photos: Pineda, M. 2023.

Figures captions

Figure 1. Parental longevity of *Drosophila suzukii* (F_0) exposed to sublethal concentrations (LC_0, LC_5, $LC_{10,}$ and LC_{20}) of spinosyn insecticides. A. Females) and B. Males, exposed to spinetoram; C. Females and D. Males exposed to spynosad.

Figure 2. Fresh weight of *Drosophila suzukii* adults exposed to low concentrations (LC_0, LC_5, LC_{10}, and LC_{20})of spinosyns. A. Pupae. B. Adults spinetoram and C. Adults spinosad.

Figure 3. Total number A-D. Number of females; B-E. Males; C-F. Total of emerged progeny flies of 5-7 days old mated *Drosophila suzukii* exposed to low concentrations (LC_0, LC_5, LC_{10}, and LC_{20}) of spinosyns.

Figure 4. . Number of oviposition scars per pupae. of *D. longicaudata* adultos survivors 72 hours, of exposure to lethal LC_{60} and sublethal LC_{10} and LC_{20}. concentrations of spinosyns.

Figure 5. Progeny of *D. longicaudata* when parents were exposed to concentrations LC_{10}, $LC_{20,}$ and LC_{60} of spinosyns. A. Total emergency; B. Sex ratio; C. Parasitism total**.**

Figure 6. Progeny total of *D. longicaudata* when parents were exposed to concentrations LC_{10}, $LC_{20,}$ and LC_{60} of spinosyns. A. Emergency rate spinetoram; B. Emergency rate spinosad.

Figure 7. Total emergence of *D. longicaudata* when parents were exposed to LC_{10}. LC_{20} and LC_{60} concentrations of spinosyns. A-C. Daily emergence of females and males; E-G. Cumulative emergence of females and males. exposed to spinetoram. B-D. Daily emergence of females and males; F-H. Cumulative emergence of females and males, exposed to spinosad.

Figure 1

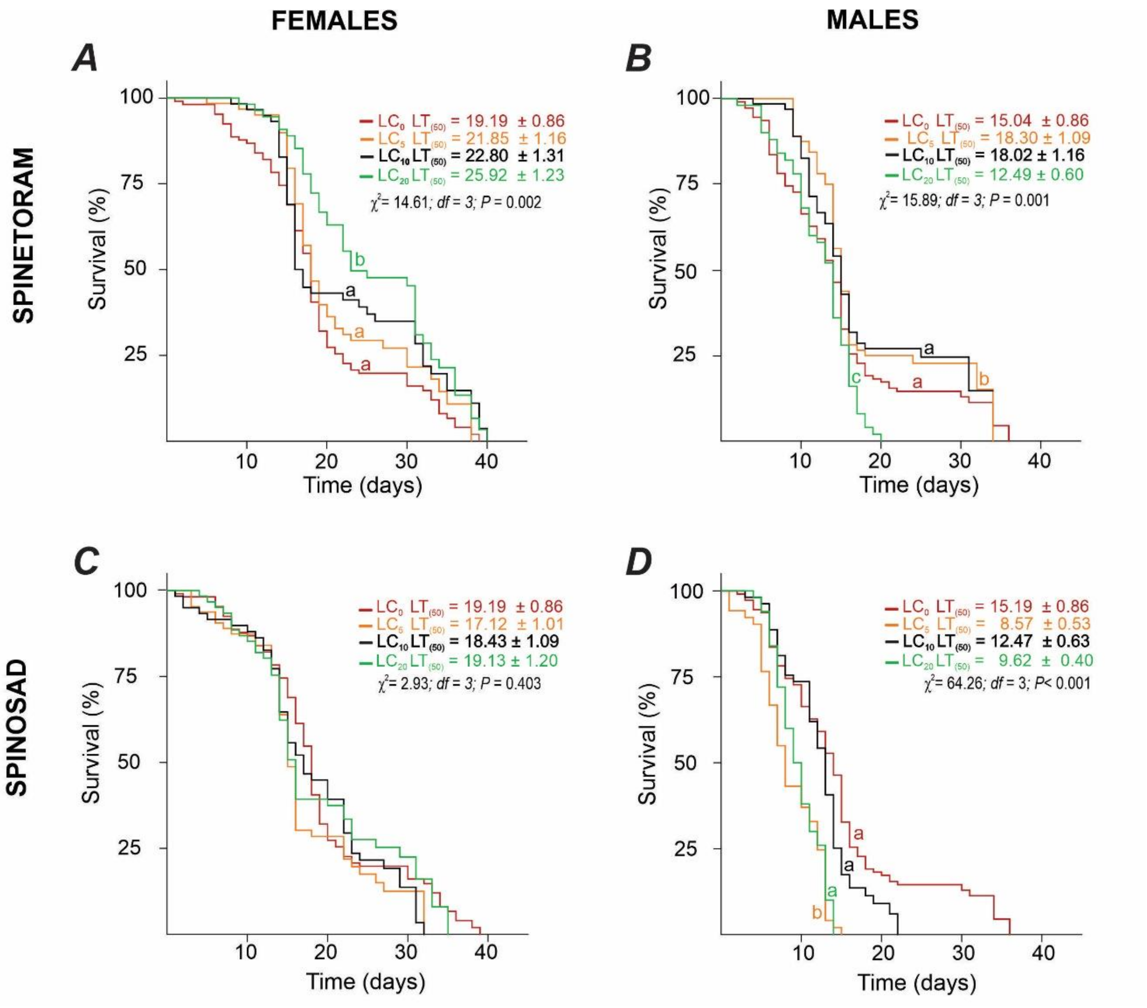
FEMALES
MALES
SPINETORAM
SPINOSAD
A
B
C
D
Survival (%)
Time (days)
100
75
50
25
10
20
30
40
LC_0 $LT_{(50)}$ = 19.19 ± 0.86
LC_5 $LT_{(50)}$ = 21.85 ± 1.16
LC_{10} $LT_{(50)}$ = 22.80 ± 1.31
LC_{20} $LT_{(50)}$ = 25.92 ± 1.23
χ^2= 14.61; *df* = 3; *P* = 0.002
b
a
a
a
LC_0 $LT_{(50)}$ = 15.04 ± 0.86
LC_5 $LT_{(50)}$ = 18.30 ± 1.09
LC_{10} $LT_{(50)}$ = 18.02 ± 1.16
LC_{20} $LT_{(50)}$ = 12.49 ± 0.60
χ^2= 15.89; *df* = 3; *P* = 0.001
a
b
c
a
LC_0 $LT_{(50)}$ = 19.19 ± 0.86
LC_5 $LT_{(50)}$ = 17.12 ± 1.01
LC_{10} $LT_{(50)}$ = 18.43 ± 1.09
LC_{20} $LT_{(50)}$ = 19.13 ± 1.20
χ^2= 2.93; *df* = 3; *P* = 0.403
LC_0 $LT_{(50)}$ = 15.19 ± 0.86
LC_5 $LT_{(50)}$ = 8.57 ± 0.53
LC_{10} $LT_{(50)}$ = 12.47 ± 0.63
LC_{20} $LT_{(50)}$ = 9.62 ± 0.40
χ^2= 64.26; *df* = 3; *P*< 0.001
a
a
a
b

Figure 2

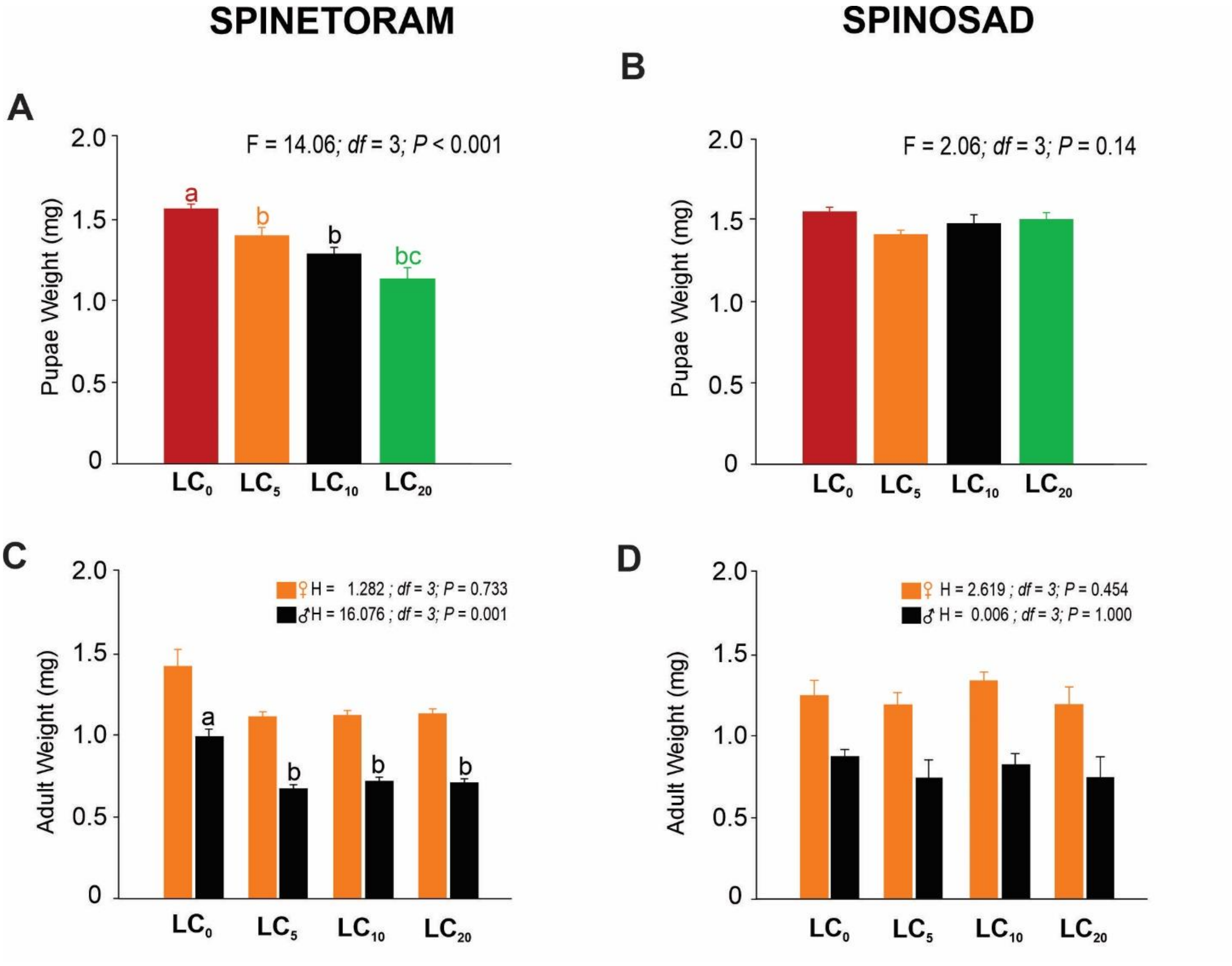
SPINETORAM
SPINOSAD
A
F = 14.06; df = 3; P < 0.001
Pupae Weight (mg)
2.0
1.5
1.0
0.5
0
a
b
b
bc
LC₀ LC₅ LC₁₀ LC₂₀
B
F = 2.06; df = 3; P = 0.14
Pupae Weight (mg)
LC₀ LC₅ LC₁₀ LC₂₀
C
♀ H = 1.282 ; df = 3; P = 0.733
♂ H = 16.076 ; df = 3; P = 0.001
Adult Weight (mg)
a
b
b
b
LC₀ LC₅ LC₁₀ LC₂₀
D
♀ H = 2.619 ; df = 3; P = 0.454
♂ H = 0.006 ; df = 3; P = 1.000
Adult Weight (mg)
LC₀ LC₅ LC₁₀ LC₂₀

Figure 3

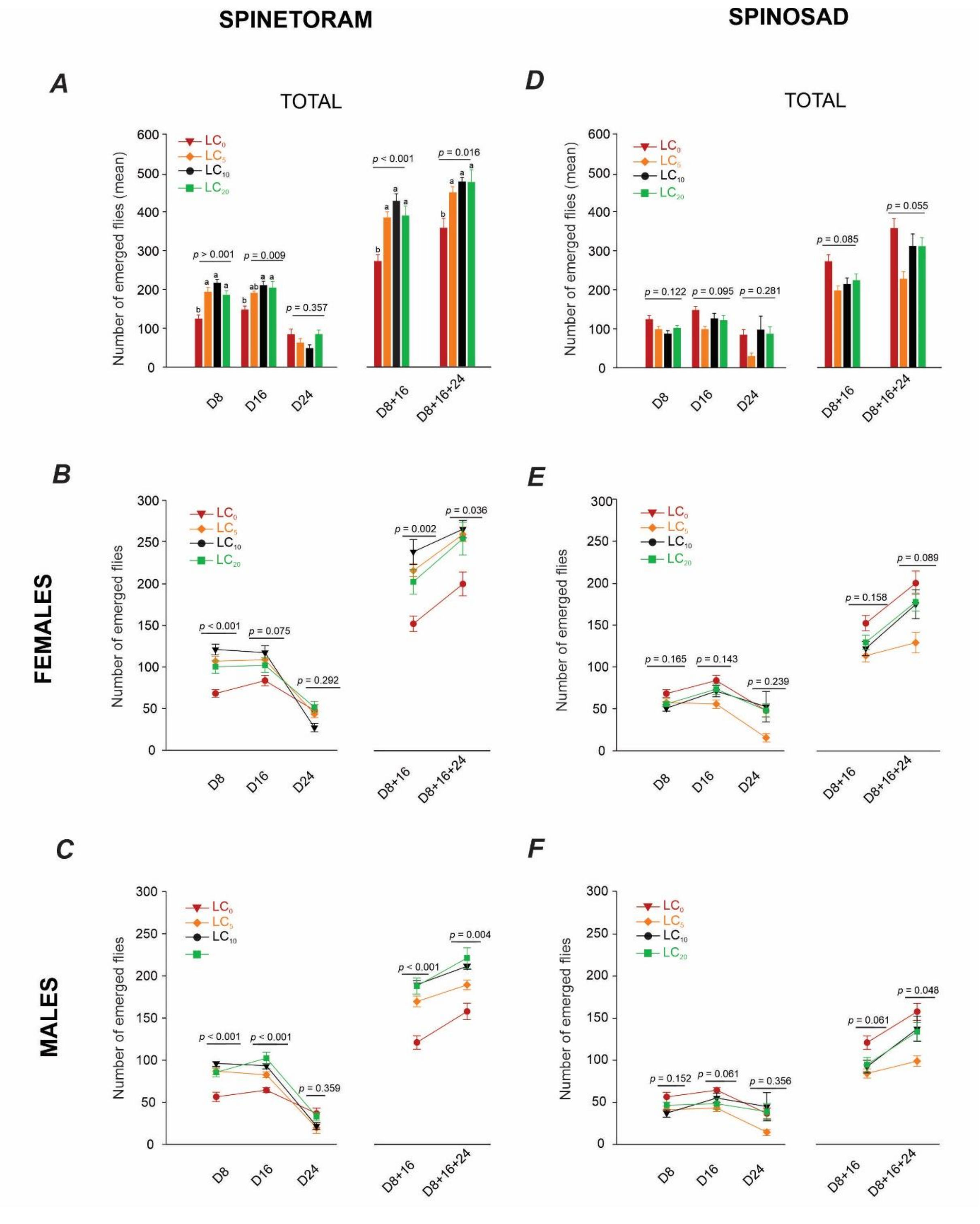
SPINETORAM
SPINOSAD
A
TOTAL
D
TOTAL
B
E
C
F
FEMALES
MALES
LC0
LC5
LC10
LC20
Number of emerged flies (mean)
Number of emerged flies
0
50
100
150
200
250
300
400
500
600
D8
D16
D24
D8+16
D8+16+24
p > 0.001
p = 0.009
p = 0.357
p < 0.001
p = 0.016
a
b
ab
p = 0.122
p = 0.095
p = 0.281
p = 0.085
p = 0.055
p < 0.001
p = 0.075
p = 0.292
p = 0.002
p = 0.036
p = 0.165
p = 0.143
p = 0.239
p = 0.158
p = 0.089
p < 0.001
p < 0.001
p = 0.359
p < 0.001
p = 0.004
p = 0.152
p = 0.061
p = 0.356
p = 0.061
p = 0.048

Figure 4

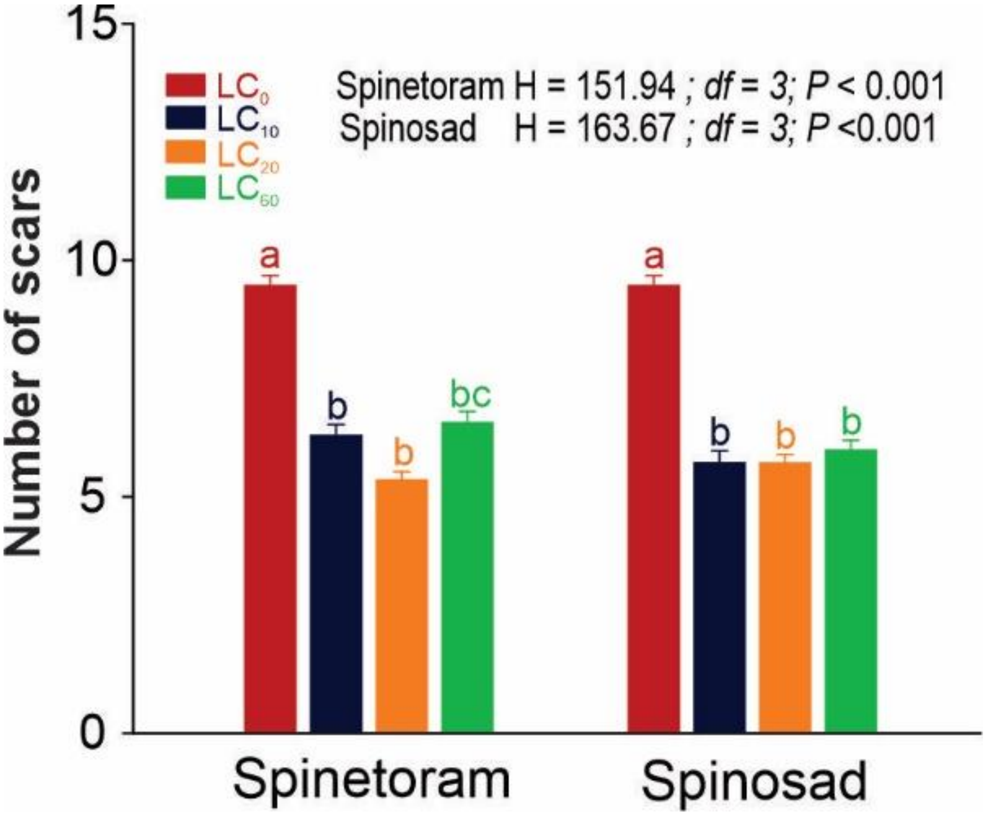
15
10
5
0
Number of scars
LC0
LC10
LC20
LC60
Spinetoram H = 151.94 ; df = 3; P < 0.001
Spinosad H = 163.67 ; df = 3; P <0.001
a
b
b
bc
a
b
b
b
Spinetoram
Spinosad

Figure 5

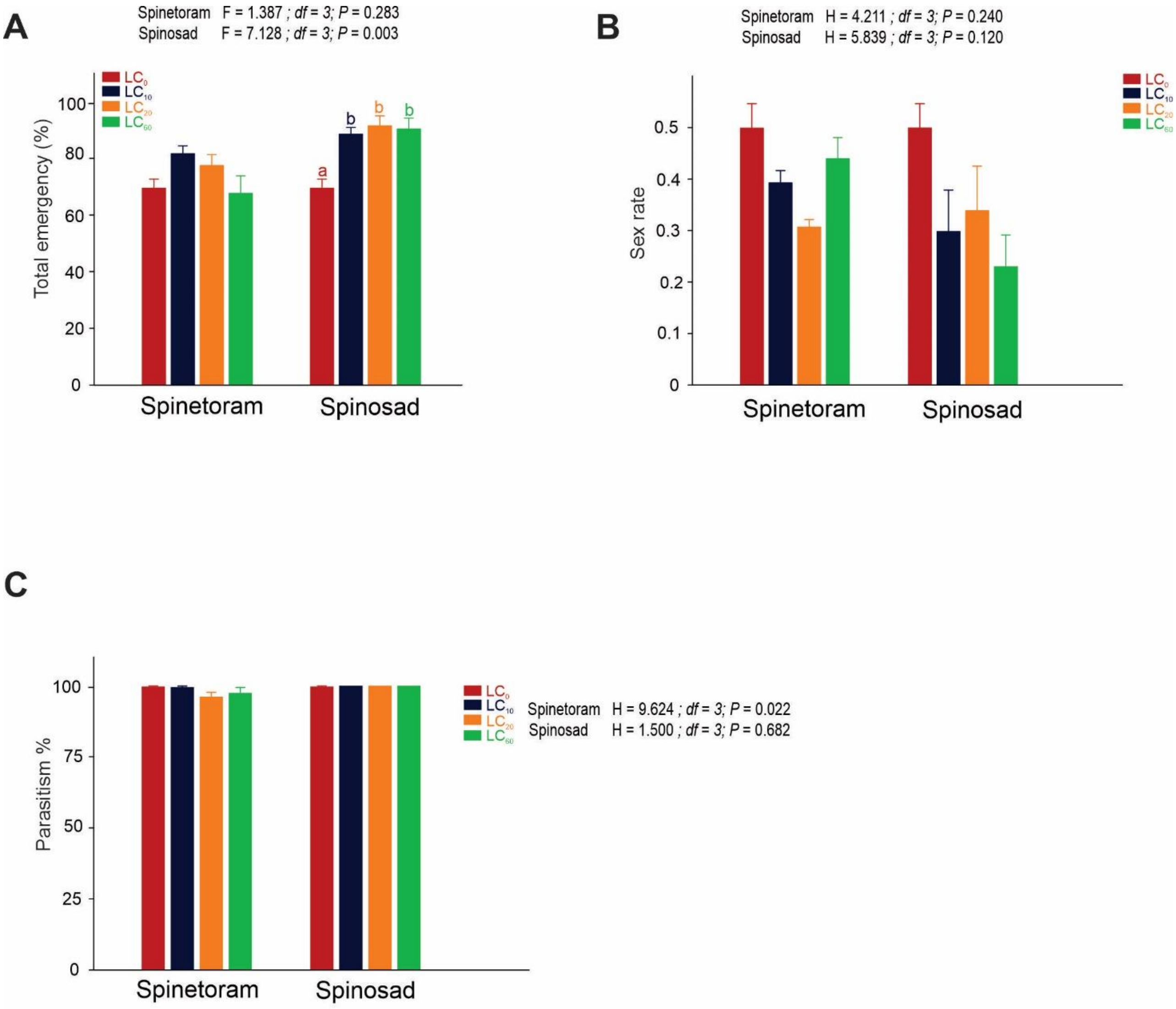
A
Spinetoram F = 1.387 ; df = 3; P = 0.283
Spinosad F = 7.128 ; df = 3; P = 0.003
LC_0
LC_{10}
LC_{20}
LC_{60}
Total emergency (%)
0
20
40
60
80
100
a
b
b
b
Spinetoram
Spinosad
B
Spinetoram H = 4.211 ; df = 3; P = 0.240
Spinosad H = 5.839 ; df = 3; P = 0.120
LC_0
LC_{10}
LC_{20}
LC_{60}
Sex rate
0
0.1
0.2
0.3
0.4
0.5
Spinetoram
Spinosad
C
Parasitism %
0
25
50
75
100
LC_0
LC_{10}
LC_{20}
LC_{60}
Spinetoram H = 9.624 ; df = 3; P = 0.022
Spinosad H = 1.500 ; df = 3; P = 0.682
Spinetoram
Spinosad

Figure 6

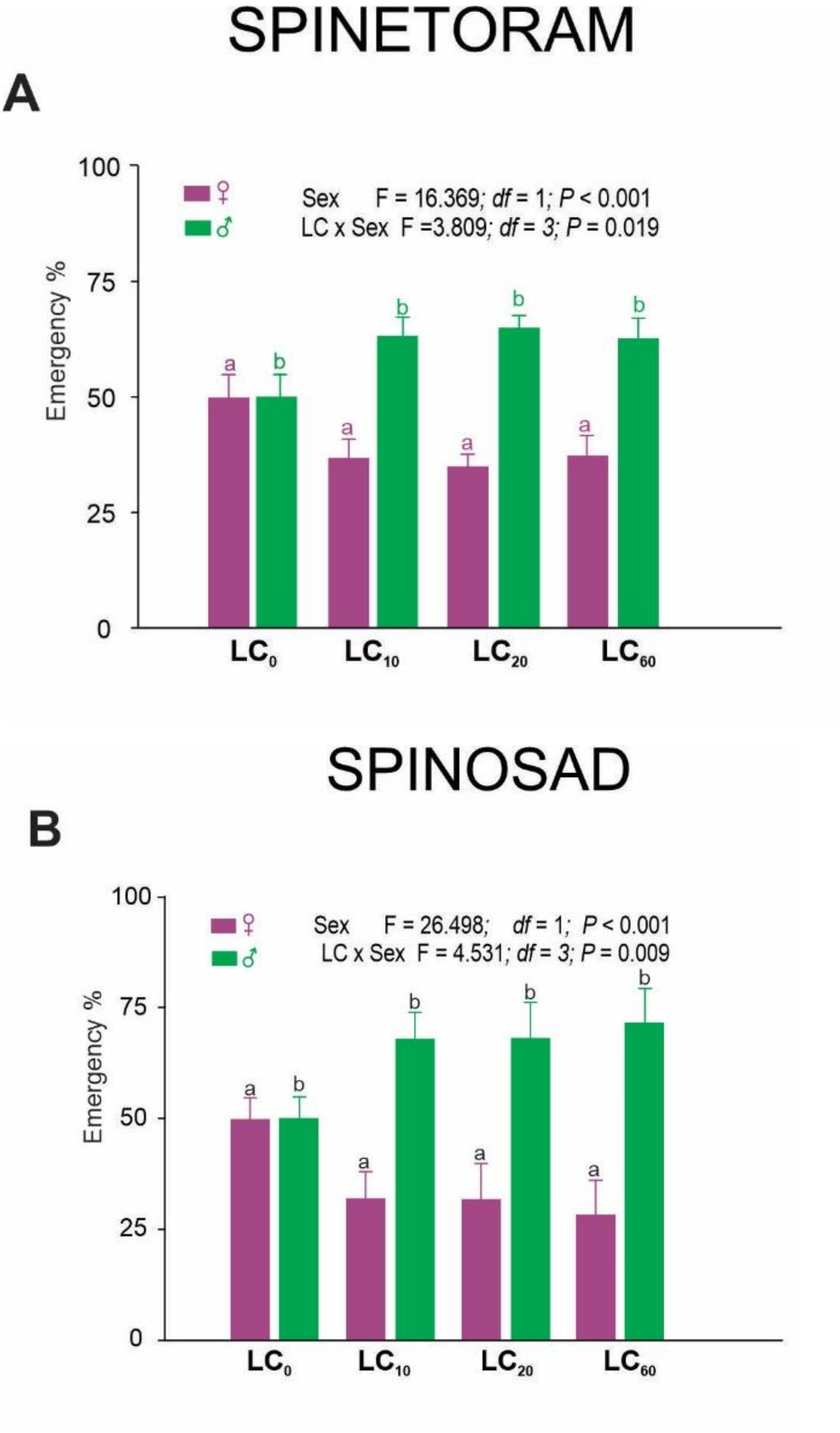
SPINETORAM
A
Emergency %
100
75
50
25
0
♀
♂
Sex F = 16.369; df = 1; P < 0.001
LC x Sex F =3.809; df = 3; P = 0.019
a
b
a
b
a
b
a
b
LC0
LC10
LC20
LC60
SPINOSAD
B
Emergency %
100
75
50
25
0
♀
♂
Sex F = 26.498; df = 1; P < 0.001
LC x Sex F = 4.531; df = 3; P = 0.009
a
b
a
b
a
b
a
b
LC0
LC10
LC20
LC60

Figure 7

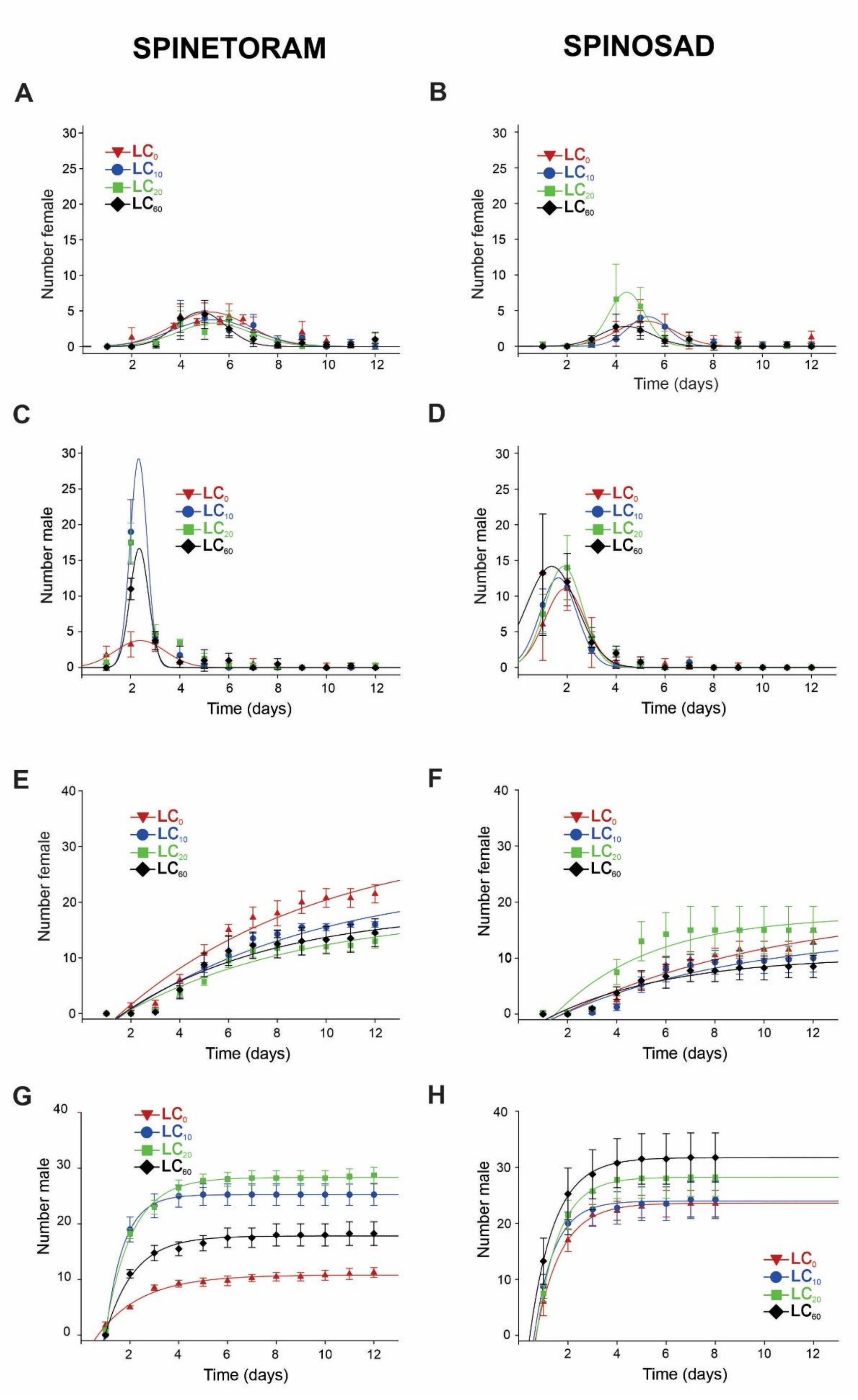
SPINETORAM
SPINOSAD
A
B
C
D
E
F
G
H
Number female
Number male
Time (days)
LC$_{0}$
LC$_{10}$
LC$_{20}$
LC$_{60}$
0
5
10
15
20
25
30
40
2
4
6
8
10
12

Table 3. Summary of the nonlinear regression analyses of the curve shown in Fig. 7.

Concentration insecticide	Estimated parameters *	Daily emergency Males spinetoram (Fig. 7C)			Daily emergency Males spinosad (Fig. 7D)		
		Value (95% CI)	*t*- value	*P*	Value (95% CI)	t- value	*P*
LC_0	a	3.791 (3.119-4.463)	12.766	<0.0001	11.026 (10.287-11.765)	33.753	<0.0001
LC_{10}		29.266 (-187.841-246.373)	0.3005	0.7673	12.566 (11.613-13.519)	29.834	<0.0001
LC_{20}		19.122 (12.909-25.334)	6.963	<0.0001	14.161 (12.772-15.551)	23.051	<0.0001
LC_{60}		16.719 (-64.711-98.150)	0.464	0.6533	14.174 (12.901-15.446)	25.194	<0.0001
LC_0	b	1.029 (0.806-1.251)	10.459	<0.0001	0.826 (0.760-0.892)	28.392	<0.0001
LC_{10}		0.346 (1.058-1.749)	0.557	0.5912	0.757 (0.678-0.835)	21.903	<0.0001
LC_{20}		0.479 (0.287-0.671)	5.64	0.0003	0.761 (0.675-0.848)	19.862	<0.0001
LC_{60}		0.378 (-0.786-1.542)	0.735	0.481	1.039 (0.831-1.248)	11.282	<0.0001
LC_0	X_0	2.383 (2.173-2.593)	25.653	<0.0001	1.908 (1.841-1.975)	64.462	<0.0001
LC_{10}		2.321 (0.868-3.774)	3.614	0.0056	1.644 (1.584-1.704)	61.985	<0.0001
LC_{20}		2.202 (1.949-2.456)	19.672	<0.0001	1.853 (1.761-1.945)	45.541	<0.0001
LC_{60}		2.346 (1.398-3.294)	5.597	0.0003	1.367 (1.171-1.563)	15.774	<0.0001
		Accumulated emergency Males spinetoram (Fig. G)			Accumulated emergency Males spinosad (Fig. 7H)		
		Value (95% CI)	*t*- value	*P*	Value (95% CI)	*t*- value	*P*
LC_0	Y_0	4.961 (-7.881 -2.042)	-3.844	0.0039	23.369 (-27.301 -19.437)	-13.445	<0.0001
LC_{10}		74.090 (-79.627 -68.552)	-30267	<0.0001	28.546 (-41.151 -15.942)	-5.123	0.0006
LC_{20}		40.562 (-47.543 -33.581)	-13.144	<0.0001	34.682 (-37.580 -31.784)	-27.076	<0.0001
LC_{60}		23.565 (-30.551 -16.579)	-7.631	<0.0001	18.036 (-21.519 -14.552)	-11.712	<0.0001
LC_0	a	15.767 (13.006-18.527)	12.919	<0.0001	46.986 (43.115-50.856)	27.46	<0.0001
LC_{10}		99.347 93.846-104.848	40.852	<0.0001	52.531 (40.035-65.027)	9.51	<0.0001
LC_{20}		68.899 (62.044-75.755)	22.736	<0.0001	62.925 (60.061-65.789)	49.7	<0.0001
LC_{60}		41.393 (34.556- 48.229)	13.696	<0.0001	49.764 (46.333-53.195)	32.812	<0.0001
LC_0	b	0.579 (0.505-0.653)	17.673	<0.0001	0.375 (0.348-0.402)	31.5	<0.0001
LC_{10}		0.254 (0.241-0.267)	43.271	<0.0001	0.289 (0.225-0.352)	10.274	<0.0001
LC_{20}		0.398 (0.364-0.432)	26.572	<0.0001	0.329 (0.316-0.343)	65.789	<0.0001
LC_{60}		0.426 (0.367-0.484)	16.384	<0.0001	0.370 (0.348-0.392)	37.505	<0.0001

* Coefficients from the three parameters log-normal model $y = \frac{a}{x} \exp\left[-0.5\left(\frac{Inx/c}{b}\right)^2\right]$. The Parameters characterize attributes of the curves. where a is the maximum value of dependent variable. b is the localitation of the peak response value on the time axis and c is the skewness (or rate of change) of the response as a function of time. Parameter values followed by different letters in the columns were significantly different (based on non-overlapping of confidence limits)

Table 4. Summary of the nonlinear regression analyses of the curve shown in Fig. 7.

Concentration insecticide	Estimated parameters *	Daily emergency Females spinetoram (Fig. 7A)			Daily emergency Females spinosad (Fig. 7B)		
		Value (95% CI)	*t*- value	*P*	Value (95% CI)	t- value	*P*
LC_0	a	4.836 (3.391-6.281)	7.571	<0.0001	3.530 (2.426-4.635)	7.231	<0.0001
LC_{10}		3.745 (2.492-4.997)	6.761	<0.0001	4.153 (3.559-4.747)	15.817	<0.0001
LC_{20}		3.287 (2.132-4.442)	6.44	0.0001	7.578 (6.668-8.487)	18.842	<0.0001
LC_{60}		4.840 (3.748-5.932)	10.027	<0.0001	2.785 (2.044-3.526)	8.505	<0.0001
LC_0	b	1.556 (0.237 -6.554)	6.554	0.0001	1.180 (0.754-1.607)	6.263	0.0001
LC_{10}		1.663 (1.020-2.306)	5.581	0.0002	0.820 (0.682-0.958)	13.476	0.0001
LC_{20}		1.500 (0.891-2.108)	5.576	0.003	0.736 (0.625-0.847)	15.018	0.0001
LC_{60}		(1.023 0.757-1.289)	86.999	<0.0001	1.041 (0.721-1.362)	7.35	0.0001
LC_0	X_0	5.240 (4.703-5.777)	22.08	<0.0001	5.217 (4.790-5.643)	27.679	<0.0001
LC_{10}		5.334 (4.692-5.977)	18.787	<0.0001	5.307 (5.174-5.440)	90.314	<0.0001
LC_{20}		5.380 (4.772-5.989)	20.011	<0.0001	4.445 (4.358-4.531)	115.763	<0.0001
LC_{60}		4.836 (4.570-5.103)	41.007	<0.0001	4.415 (4.096-4.734)	31.286	<0.0001
		Accumulated emergency Females spinetoram (Fig. 7E)			Accumulated emergency Females spinosad (Fig. 7F)		
		Value (95% CI)	*t*- value	*P*	Value (95% CI)	*t*- value	*P*
LC_0	Y_0	6.702 (-12.304-1.100)	-2.706	0.0241	4.266 (-8.291 -0.242)	-2.398	0.04
LC_{10}		5.788 (-10.895 -0.681)	-2.564	0.0305	3.988 (-8.510 -0.534)	-1.995	0.0772
LC_{20}		4.804 (-9.403 -0.204)	-2.363	0.0424	8.197 (-17.421 -1.027)	-2.01	0.0753
LC_{60}		(5.783 (-11.436 -0.130)	-2.314	0.0459	3.800 (-6.959 -0.641)	-2.721	0.0236
LC_0	a	38.699 (25.143-52.256)	6.458	0.0001	23.821 (11.747-35.896)	4.463	0.0016
LC_{10}		30.428 (18.117-42.740)	5.591	0.0003	18.602 (10.096-27.107)	4.497	0.0008
LC_{20}		23.124 (14.741-31.506)	6.24	0.0002	25.772 (18.451-33.093)	7.964	<0.0001
LC_{60}		24.361 (17.549-31.172)	8.09	0.0459	14.092 (11.452-16.731)	12.077	<0.0001
LC_0	b	0.886 (0.791-0.982)	20.948	<0.0001	0.895 (0.777-1.013)	17.175	<0.0001
LC_{10}		0.886 (0.775-0.997)	18.08	<0.0001	0.875 (0.724-1.027)	13.075	<0.0001
LC_{20}		0.874 (0.751 - 0.997)	16.069	<0.0001	0.775 (0.608-0.942)	10.503	<0.0001
LC_{60}		0.852(0.721-0.982)	14.748	<0.0001	0.816 (0.703-0.929)	16.293	<0.0001

* Coefficients from the three parameters log-normal model $y = \frac{a}{x}\exp\left[-0.5\left(\frac{Inx/c}{b}\right)^2\right]$. The Parameters characterize attributes of the curves. where a is the maximum value of dependent variable. b is the localitation of the peak response value on the time axis and c is the skewness (or rate of change) of the response as a function of time. Parameter values followed by different letters in the columns were significantly different (based on non-overlapping of confidence limits)

Supplementary Figure 2

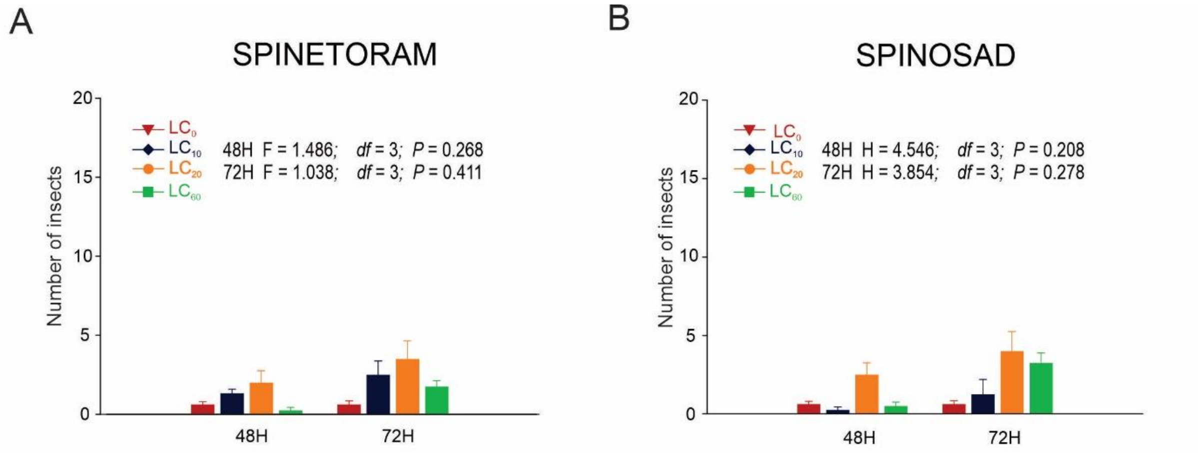

Figure 2. Total mortality after 24 and 48 hours of *Diachasmimorpha longicaudata* low concentrations (Lc$_0$, LC$_{10}$,LC$_{20}$ and LC$_{60}$) of spinosyns: A. spinetoram; B. spinosad.

Supplementary figure 3

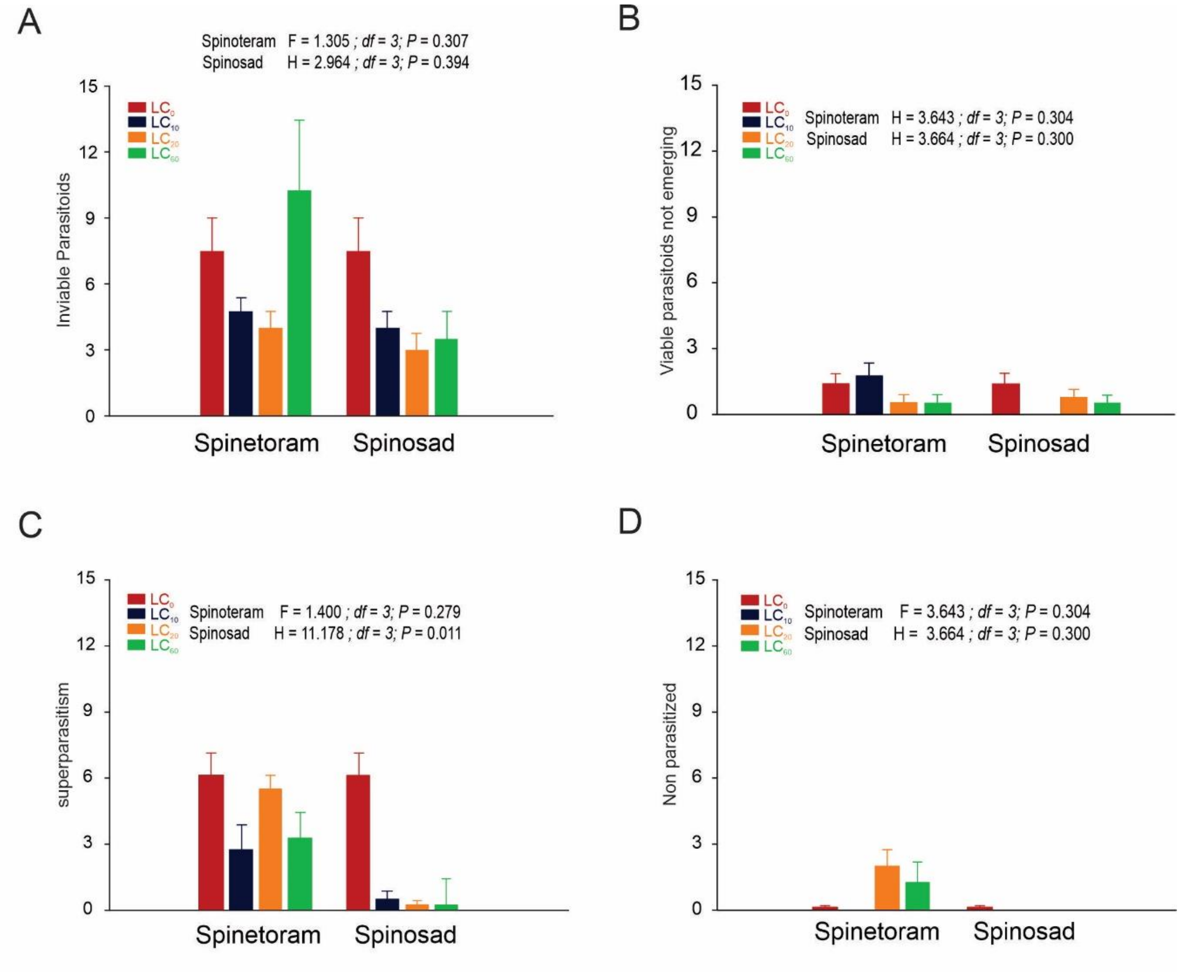

Figure 3. Offsping of *Diachasmimorpha longicaudata* exposed to concentrations (Lc$_0$, LC$_{10}$,LC$_{20}$ and LC$_{60}$) of spinosyns: **A.** Inviable parasitoids; **B.** Viable parasitoids not emerging; **C.** Superparasitismo; **D.** Non parasitized.

ARTICLE III

Toxic effect of orange essential oil *Citrus sinensis* (L.) Osbeck and its major component (R)-(+)-Limonene on adults of *Drosophila suzukii*

Enviromental Entomology Journal standards (preliminary version)

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Abstract

Integrated management of *Drosophila zusukii* is based on the use of synthetic insecticides, mainly from the spinosyns group. Considering that spotted wing drosophila is an exotic pest that can cause great damage to Brazilian fruit growing, it is necessary to seek new strategies for its control. In recent years, the use of natural products has aroused much interest in the use of essential oils and their major components for the control of agricultural pests. In addition to having insecticidal properties, these products are environmentally friendly, resistance has not yet been reported and they can be included in IPM. However, most research focuses mainly on their lethal activity, and side effects are underestimated. Thus, our first objective was to determine the dose-response curve of sweet orange essential oil *Citrus sinensis* (L.) Osbeck and its major component (R)-(+)-Limonene in 7-day-old adults. We subsequently selected low concentrations (Control LC_0, LC_1, LC_5, LC_{10}; LC_{20}) of both the essential oil and the major compound, and 7-day-old adult flies were exposed for 48 hours. We determined the longevity of exposed adults, as well as the fertility, and weight of pupae and adult offspring. Our results show that orange EO is more toxic than its major compound, although both showed insecticidal activity for control of adult *D. suzukii* flies. On the other hand, it was observed that at low doses of the essential oil the longevity of exposed adults was prolonged, and the opposite effect occurred when exposed to limonene. In both cases, total fertility was affected. Our results demonstrate that the use of EOs is an optimal alternative for the control of adult flies, there are hormetic responses when exposed to low concentrations, and further studies should be developed for other life stages of the insect, as well as the type of formulation.

Keywords: Bioinsecticide, Hormesis, Spotted wing drosophila, Toxicity, Low-concentration.

Introduction

The use of synthetic insecticides is considered the most effective strategy for controlling agricultural pests compared to other alternatives. However, the misuse of pesticides, along with other factors such as climatic variables (Deutsch et al. 2018), has changed this situation, causing pests to develop resistance to chemical molecules, resulting in less effective pest control (Matzrafi 2019, Pu et al. 2020). In addition to accumulating in the environment, pesticide residues can cause various problems, including human health problems, loss of natural predators, and contamination of water, soil, and food. These residues can indirectly affect target pests by creating stimuli that alter the biology and reproduction of the species. This phenomenon is called the hormetic effect (Papanastasiou et al. 2017, Tjaden et al. 2018, Haddi et al. 2020, Guedes et al. 2022). As a result, researchers are exploring alternatives to conventional pesticides for pest control, leading to a growing interest in natural-source pesticides. One of the most effective alternatives is the use of essential oils that have insecticidal properties and can fit into IPM strategies(Koul et al. 2008). Essential oils (EOs) are volatile substances extracted from various plant parts, mainly flower, leaf, and root (O'Bryan et al. 2015). They have a low molecular weight and are composed of alkaloids, tannins, steroids, glycosides, resins, phenols, essential oils, and flavonoids (Asgari Lajayer et al. 2017).

Essential oils have historically been used in medicine, perfumery, cosmetology, and the culinary industry (Bolouri et al. 2022). However, as they are considered safer and more environmentally friendly than synthetic insecticides(Koul et al. 2008), research into their insecticidal properties has intensified in recent years. The best-known mechanisms of action of EOs act as neurotoxicants and repellents. In many cases, the visible symptoms of the insecticidal activity of EOs are hyperactivity, convulsions, tremors, and paralysis (Shaaya and Rafaeli 2007). Nevertheless, the mechanisms of toxic action of ECs have not been fully elucidated to date. *Citrus,* a genus in the Rutaceae family, is the most widely cultivated fruit in the world (Moore 2001). Due to its chemical composition of secondary metabolites, *C. sinensis* has been the subject of extensive research by the pharmaceutical industry. Numerous chemical compounds have been discovered in the fruit, peel, leaves, juice, and roots of C. *sinensis.*, including flavonoids, steroids, hydroxyamides, alkanes, fatty acids, coumarin, peptides, carbohydrates, alkylamines, carotenoids, and other volatile compounds. These substances have various applications, such as fungicides, antibacterials, acaricides, nematicides, and insecticides (Kelebek and Selli 2011, Favela-Hernández et al. 2016). These biological activities are often ascribed to the existence of compounds like α-pinene, β-pinene, β-myrcene, and predominantly Limonene, which is its major compound and typically comprises more than 80% (v/v). These compounds have demonstrated lethality towards several insect pest species, including *Aedes aegypti*, *Sitophilus zeamais*, and *Drosophila suzukii* (Lourenço et al. 2018, de Souza et al. 2022a, Wangrawa et al. 2022, Fouad et al. 2023). Nonetheless, the impact on organisms exposed to low concentrations remains unknown.

Drosophila suzukii (Matsumura, 1931), the spotted wing fly, of Asian origin, entered Brazil in 2013 (Deprá et al. 2014). An exotic pest with a preference for red fruits. Considering that Brazil is the third largest fruit-producing country in the world, its importance lies in the oviposition capacity of the females inside healthy fruits, because it has a sclerotized and sawed ovipositor. The larvae develop inside the fruit by feeding on the pulp, making them lose their commercial value. The pupal stage usually occurs on the ground, from where the adults emerge again to complete a new cycle. It has a short life cycle of approximately 10-12 days at an average temperature of 25ºC. Integrated management of this pest is mainly based on cultural practices and the use of synthetic insecticides such as spinosyns (Andreazza et al. 2016, Schlesener et al. 2018, Gress and Zalom 2019).

Therefore, in this research we investigated the lethal effect and after-effects of adult flies *D. suzukii* exposed to low concentrations of sweet orange EO *Citrus sinensis* (L.) Osbeck and its major component Limonene. We documented the response in adult longevit, and the body mass of pupae and adult progeny.

Material and Methods

Essential Oil-EO

The EO of *Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae) was commercially purchased in 10 ml sealed amber bottles from WNF Indústria e Comercio Ltda (WFN 2022). The major component (R)-(+)-Limonene 97% was acquired from Sigma-Aldrich warrants.

Chemical characterization

Furthermore, the chemical identification of the constituents was performed by gas chromatography coupled to mass spectrometry (GC-MS), using a model QP 2010 Plus equipment (Shimadzu Corporation, Kyoto, Japan) operating with a capillary column of fused silica (30 m × 0.25 mm) with a DB-5 bound phase (film thickness, 0.25 µm). Helium was used as carrier gas at a flow of 1.0 mL min^{-1}. Injector and detector temperatures were 220 and 240 ˚C, respectively. The sample injection volume was 0.5 µL, diluted in hexane (1%) (Sigma-Aldrich®, St. Louis, MO, USA) and injection volume partition ratio (split) of 1:100. Temperature ramp started at 60 ˚C, with an increase at a rate of 3 ˚C min^{-1} to 240 ˚C, followed by an increase at 10 ˚C min^{-1} until reaching 300 ˚C, with the final temperature maintained for 7 min. Column pressure was around 71.0 kPa. The mass spectrometer was operated with an ionization potential of 70 eV and an ion source temperature of 200 ˚C. The mass analysis was performed in full-scan mode, ranging from 45 to 500 Da, with a scan speed of 1000 Da s^{-1} and a scanning interval of 0.5 fragments s^{-1}. Data were obtained and processed using the Lab Solutions LC/GC Workstation 2.72 software (Shimadzu, Kyoto, Japan). The retention index of the compounds was calculated in relation to a homologous series of n-alkanes (nC_9–nC_{18}), using the equation of Van den Dool and Kratz (van Den Dool and Dec. Kratz 1963). The identification of compounds was performed by comparing the calculated retention indices with those described in the literature(Adams 2007). Comparisons of the mass spectra obtained with those existing in the FFNSC 1.2, NIST107 and NIST21 libraries were also performed. Quantitative analysis was performed by gas chromatography with a flame ionization detector (GC-FID), using model GC-2010 equipment (Shimadzu Corporation, Kyoto, Japan), with experimental conditions identical to those used in the qualitative analysis, except the detector temperature, which was 300˚C and acetylene was used as carrier gas. The relative percentages of each constituent were obtained by the area normalization method.

Bioassay of essential oils toxicity to *Drosophila suzukii*

Bioassays of concentration-mortality were carried out on the essential oil of orange and its main constituent was conducted to determine the contact and ingestion of lethal activity *D. suzukii* adults. The colony of *D. suzukii*, used in the experiments is 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). Adult exposure was performed according to IRAC (Insecticide Resistance Action Committee -IRAC) protocol

No.26 with adults of *Musca domestica* L. (Diptera: Muscidae), with slight modifications described below (IRAC 2006, de Souza et al. 2022). Firstly, 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, seven concentrations within it were used to determine the dose-response curve. The serial concentrations were prepared by diluting to the final concentration the corresponding EO volumes in dimethyl sulfoxide DMSO (2.5% $^{v}/_{v}$) and a sugar solution (20% $^{w}/_{v}$). 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. The negative control, was the DMSO (2.5% $^{v}/_{v}$) in sugar solution (20% $^{w}/_{v}$). Subsequently, for each repetition, 20 to 25 non-sexed flies of the same age (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 hours of exposure. Flies were considered dead if they did not show any movement even after being podded with a fine brush.

Exposure of *D. suzukii* adults' to essential oils low concentrations

After determining the dose-response curve of orange essential oil and its main compound limonene, the following concentrations were selected: LC_0 (control water + DMSO), LC_1, LC_5, LC_{10} 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, following the methodology of Pineda et al., (2023). After 48 hours, mortality was evaluated and the survivors were transferred to new containers with sugar solution (20% $^{w}/_{v}$). After 24 hours, 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 (for one liter of water: 67 g sugar, 23 g brewer's yeast, 42 g corn flour, 15 g agar, 5.9 ml propionic acid and 10% nipagin), with five repetitions for each concentration (Pineda et al. 2023; Andreazza et al. 2016a). The daily mortality of the parents was evaluated, and every 8 days the survivors were transferred to new glass with diet, in order to avoid a clash between the generations. For three weeks, the number of flies emerging daily was counted. Additionally, the body weight of *D. suzukii* pupae and emergent adults was estimated. Fifty pupae and Fifty adults (female and male, separately) from each concentration were determinate.

Statistical analyisis

Dose-mortality data were subjected to 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 that emerged, 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 pairwaise multiple comparison analysis of means Holm-Sidak method; Tukey's analysis or Dunn's Method ($p \leq 0.05$).

Results

Chemical characterization of the essential oil

The chemical characterization showed that six different monoterpenes were present. The main component was Limonene (95.29%), followed by β-pinene (1.29%), ϓ-terpinene (1.22%), p-cymene (0.94%), myrcene (0.75%) and α-pinene (0.51%) (Table 1).

Toxicity bioassays

The mean lethal concentrations (LC_{50}) estimated for the essential oil, and main constituents against adult's *D. suzukii* are presented in Table 2. The lethal concentration LC_{50}, indicated that orange EO (0.33 µl.mL^{-1}) was more toxic than its major component Limonene (8.12 µl.mL^{-1}).

Effects of low concentrations of *D. suzukii* flies

Adults' Longevity

Longevity (LT_{50}) of *D. suzukii* adults exposed to sweet orange essential oil had a longer mean lifespan when exposed to limonene. Thus, the essential oil of orange showed significant differences in the mean life span of the females and males exposed at the following concentrations tested LC_0, LC_1, LC_5, LC_{10} and LC_{20} (♀: $\chi^2 = 11.395$, *df* = 4, *p*= 0.022; ♂: $\chi^2 = 38.254$, *df* = 4, *p* <0.001) (Figures 1A-C). In both cases, there was an increase in the average life expectancy of adults with LC_{20} (♀LT_{50}= 21.841 ± 1.559 d; ♂ LT_{50}= 22.347 ± 1.822 d) and LC_1 (♀LT_{50}= 22.689 ± 1.215 d; ♂ LT_{50}= 17.061 ± 1.453 d) in comparison with the control group LC_0 (♀LT_{50}= 17.868 ± 0.903 d; ♂ LT_{50}= 12.351 ±0.879 d). In contrast, when exposed to the main compound in orange essential oil, adults of the same age were exposed to Limonene. So, in females there was no statistical difference in the half-life (♀: $\chi^2 = 1.984$, *df* = 4, *p* = 0.739) (Figures 1B)., but in males the difference was evident (♂ $\chi^2 = 31.880$, *df* = 4, *p* < 0.001) (Figures 1D), because the half-life decreased as the lethal concentration increased LC_{10} (LT_{50}= 6.968 ±0.477 d), LC_{20} (LT_{50}= 7.422 ± 0.633 d) LC_0 (LT_{50}= 12.351 ±0.879 d).

Body mass of pupae and adults

There were statistical differences in the body mass of pupae exposed to orange EO (F = 7.628; *df* = 4; *p* < 0.001) compared to those exposed to the majority component Limoneno (F = 6.870; *df* = 4; *p* = 0.143) (Figure 2A). In relation to the weight of adults from parents exposed the same treatments, the females of the adults that were exposed to limonene (H = 6.350; *df* = 4; *p* = 0.175) had a higher body mass than those that were exposed to orange essential oil (H = 12.824; *df* = 4; *p* = 0.012) (Figure 2B-C). Interestingly, the opposite effect was observed in the offspring exposed to limonene (H = 10.479; *df* = 4; *p* = 0.033), where the males had a greater body mass in relation to the females (Figure 2B-C).

Adult fertility

The effective fertility of adults exposed to orange EO and its major compound, limonene, was determined using daily offspring counts. Weekly values for each sex (D8; D16 and D24) were used for the analyses (Figure 3). Concerning the female offspring, only for the orange EO on D16 (F=3.080; *df* = 4; *p* = 0.033) (Figure 3A) there were statistical differences regarding the concentrations LC_1 (91,200 ± 10,185) and LC_5 (45,600 ± 12,848). On the other hand, in the male offspring, significant statistical differences were found in the first week D8 (F=3.653; *df* = 4; *p* = 0.017) (Figure 3C) for the EO orange and its main component Limonene (F=3.027; *df* = 4; *p* = 0.035) (Figure 3D). Finally, the analysis of variance of the total emergence (sum of females and males) showed no statistical difference in any of the cases, for the orange EO, in contrast to the offspring exposed to limonene, where a difference was detected at D16 (F=2.728; *df* = 4; *p* = 0.050) and D8+d16 (F=11.520; *df* = 4; *p* = 0.021) (Figure 3E-F).

Discussion

The insecticidal properties of orange EO and its major constituent, Limonene, have been previously reported for controlling various insect pests. However, here we show that despite its toxicological efficacy in the control of *D. suzukii* adults, when exposed at low concentrations, there are positive changes in the survival of exposed adults. In addition, the EO promoted positive responses in the weight of pupae and adult offspring, while the major compound, Limonene, had a direct effect on fertility. EOs are classified as biopesticides, according to the US Environmental Protection Agency (EPA). They are defined as products whose active ingredients are derived from natural materials. There are currently 10 products with commercial essential oil formulations, including orange peel-based PREV-AM® with 5-6% essential oil and XT-2000 Orange Oil Plus with 95% d-limonene (Isman 2020, EPA 2022, Assadpour et al. 2023). This makes EOs suitable for inclusion of Integrated Pest Management, not only because they are toxic to key insects, but also they are compatible with controlling species and, above all, environmentally friendly (Isman 2016, Andreason et al. 2018, Abubakar et al. 2020).

The insecticidal properties of orange essential oil and its primary constituent Limonene have been previously documented for the management of numerous insect pests (Isman et al. 2011). However, we show here that although it is highly toxic for the control of *D. suzukii* adult insects, at low concentrations, there are beneficial changes in the viability of exposed adults. In addition, the EO of orange showed a beneficial effect on the weight of the pupae and adult offspring, while the main compound had a direct effect on fertility. The results show that the essential oil of orange was eight times more toxic (LC_{90} = 1.004 $\mu L.mL^{-1}$) than the parent compound limonene (LC_{90} = 8.52 $\mu L.mL^{-1}$) (Table 2). It is worth mentioning that the insects were exposed mainly by ingestion, and to a lesser extent by tarsal contact. Direct ingestion of the EO or limonene ensured an efficient toxic action. Souza et al (2022) reported that this method of application of three EOs (*Illicium verum, Myristica fragrans and Schinus molle*) caused damage to various organs and tissue alterations in *D. suzukii* that were exposed. Both Limonene and EO orange have already been reported for the control of other Diptera such as (Klauck et al. 2018), *Haematobia irritans irritans, Musca domestica* (Muscidae) (Espinoza et al. 2021), *Aedes aegypti* (Palacios et al. 2009) and *Culex quinquefasciatus* (Culicidae) (Pavela et al. 2014), *Bactrocera dorsalis* (Wen et al. 2021) and *Ceratitis capitata* (Tephritidae) (Kumar et al. 2012, Benelli et al. 2021, Jaffar and Lu 2022)

Despite presenting a remarkable efficiency as an insecticide, when adults of spotted-wing *Drosophila* are exposed to low concentrations, this stressor triggers biostimulant effects that are directly related to behavioral, biological, and reproductive alterations, this phenomenon is known as hormesis (Calabrese 2016, Calabrese et al. 2018), is widely documented in insecticides. However, we demonstrated that in the case of orange essential oil when adults of *D. suzukii* were exposed to LC_1, the longevity of females and males was increased and was reflected in the fertility D8 and D16 (Figure 3A-C) (Haddi et al. 2015, 2016, Tang et al. 2019, Cutler et al. 2022, Rix et al. 2022). Interestingly, the opposite effect occurred with males exposed to low concentrations of limonene which, although they presented a shorter life span, differences in total fertility were evidenced (Figure 3A-C). Adverse effects on nutrition and reproduction of species such as *Spodoptera frugiperda* (Cruz et al. 2016), and mortality in third-instar larvae of *Aedes aegypti* when exposed to limonene(Hardstone et al. 2010, Nascimento et al. 2017) have already been reported.

Variations were evident in relation to the body weight of pupae; for example, in pupae and female offspring of flies exposed to LC_{20} of essential oil, the weight was lower and, curiously in the case of limonene, adult males presented a slight increase, when compared to the control (Figure 2A) We consider that there may be compensation or decompensation in the body mass of the offspring, resulting from intoxication at low concentrations of the essential oil and its major component. Some reviews mention that in several cases, essential oils/components can be phytotoxic at high concentrations to plants, and beneficial to plants at low concentrations (Calabrese, 2008). However, despite the evidence, there is still much research to be done regarding all the effects that hormesis can cause, as well as their mechanisms of action. The bioinsecticides such as essential oils and their major compounds have multisite action, affecting different metabolic pathways, depending on the type of application and life stage of the pest. Thus, monoterpenes are considered effective larvicide (Cheng, Chang, et al. 2009, Cheng, Huang, et al. 2009, Aciole et al. 2011, Lima et al. 2011). Many accounts mention significant physiological, biological and behavioral effects on larvae and adults of culicidae (Magalhães et al. 2010). On the other hand, histological studies demonstrated that the mesenteron histology of *A. aegyptii* larvae exposed to R-limonene exhibited an increase in epithelial cells (Calabrese 2008, Ray et al. 2009, Oliveira et al. 2021).

Recent studies confirm that histological analysis showed alterations in the epithelium and carbohydrate distribution in the midgut, as well as a reduction in the size of adipose cells and thoracic muscle fibers of *D. suzukii* exposed to low concentrations of *I. verum* essential oil (de Souza et al. 2022). In this sense, this would explain the difference in body mass between pupae and adult offspring of adults exposed to low concentrations of orange EO and limonene. The obtaining and absorption of nutrients, takes place in the midgut, which in turn is covered by the peritrophic membrane whose function is to protect the mesenteron from abrasive particles, and external infections, therefore in immature stages determines the energy reserves of adult insects (Chapman 2013, Perumalsamy et al. 2013, Nation 2022) If, limonene in lethal doses causes a process of cellular apoptosis that directly compromises the physiology and function of the insect, however, in small doses the effects can be visualized in body deformations, less adipose tissue in pupae and adults, as we demonstrated in this study.

Finally, we suggest that the accumulation of monoterpenes such as Limonene and EOs, accumulate in the epithelial cells of the intestine, and the damage is greater when the insect acquires these compounds by ingestion, interrupting the normal process of digestion and absorption of nutrients for the normal development of insects. Now, an important point that has been little researched is the most efficient way to produce products formulated with essential oils, partly due to the low physicochemical stability, high volatility, thermal decomposition and low solubility in water. Nano-formulations are an alternative that can allow more efficient uses of these biomolecules to improve physicochemical characteristics and maintain insecticidal properties (Echeverría and Albuquerque 2019, Sharma et al. 2020, Caetano et al. 2022, Gupta et al. 2023). In any case, further studies are needed to dissualidate the best functioning of the mechanisms of action, at different stages of the insect. It is also necessary to determine the best methods of application to make these biopesticides efficient.

Conclusions

Our research shows that in addition to essential oil and its main component Limonene have insecticidal properties for the control of adults of *D. suzukii*. However, when adults of D. *suzukii* were exposed to sublethal concentrations, hormetic effects were observed. In females and males, the mean lifespan increased when exposed to EO, and interestingly the opposite effect occurred in males when exposed to LC_{10} of the major compound Limonene. It was also shown that as the sublethal dose increased the weight of pupae and adult offspring of adults exposed to low concentrations of EO was lower. Finally, significantly more adults were found from the progeny of adults exposed to LC_1 of EO, while the progeny that stood out for the major compound Limonene was LC_{20}. EOs, the majority compounds can also stimulate the reproduction and half-life of spotted-wing drosophila when exposed to low concentrations. Thus, further research should be conducted to determine the mode of action, type of formulation, type of application and all possible risks they may cause, before they are included as part of integrated management program strategies.

Author Contributions M.P.: methodology, investigation, formal analysis, writing—original draft; 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.

Funding: This research was funded by the National Council of Scientific and Technological Development (CNPq), Minas Gerais State Foundation for Research Aid (FAPEMIG, APQ-02230-21), and Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Capes, Brazil) (Finance code 001).

Institutional Review Board Statement: Not applicable.

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.

References

Abubakar, Y., H. Tijjani, C. Egbuna, C. O. Adetunji, S. Kala, T. L. Kryeziu, J. C. Ifemeje, and K. C. Patrick-Iwuanyanwu. **2020**. Pesticides, History, and Classification, pp. 29–42. *In* Nat. Remedies Pest, Dis. Weed Control. Elsevier.

Aciole, S. D. G., C. F. Piccoli, J. E. Duquel, E. V. Costa, M. A. Navarro-Silva, F. A. Marques, B. L. N. S. Maia, M. L. B. Pinheiro, and M. T. Rebelo. **2011**. Insecticidal activity of three species of *Guatteria* (Annonaceae) against *Aedes aegypti* (Diptera: Culicidae). Rev. Colomb. Entomol. 37: 262–268.

Adams, R. 2007. Identification of essential oil components by gas chromatography/Quadrupole Mass Spectroscopy, Allured Pu. ed. Texensis Publishing, Gruver, TX USA.

Andreason, S. A., N. Prabhaker, S. J. Castle, F. Ganjisaffar, D. R. Haviland, B. Stone-Smith, and T. M. Perring. **2018**. Reduced Susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to Commonly Applied Insecticides. J. Econ. Entomol. 111: 2340–2348.

Andreazza, F., K. Haddi, E. E. Oliveira, and J. A. M. Ferreira. **2016**. *Drosophila suzukii* (Diptera: Drosophilidae) Arrives at Minas Gerais State, a Main Strawberry Production Region in Brazil. Florida Entomol. 99: 796–798.

Asgari Lajayer, B., M. Ghorbanpour, and S. Nikabadi. **2017**. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicol. Environ. Saf. 145: 377–390.

Assadpour, E., A. Can Karaça, M. Fasamanesh, S. A. Mahdavi, M. Shariat-Alavi, J. Feng, M. S. Kharazmi, A. Rehman, and S. M. Jafari. **2023**. Application of essential oils as natural biopesticides; recent advances. Crit. Rev. Food Sci. Nutr. 1–21.

Barkley, N. A., M. L. Roose, R. R. Krueger, and C. T. Federici. **2006**. Assessing genetic diversity and population structure in a *Citrus* germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. 112: 1519–1531.

Benelli, G., R. Rizzo, V. Zeni, A. Govigli, A. Samková, M. Sinacori, G. Lo Verde, R. Pavela, L. Cappellacci, R. Petrelli, E. Spinozzi, M. R. Morshedloo, F. Maggi, and A.

Canale. **2021**. *Carlina acaulis* and *Trachyspermum ammi* essential oils formulated in protein baits are highly toxic and reduce aggressiveness in the medfly, *Ceratitis* capitata. Ind. Crops Prod. 161: 113191.

Bolouri, P., R. Salami, S. Kouhi, M. Kordi, B. Asgari Lajayer, J. Hadian, and T. Astatkie. **2022**. Applications of Essential Oils and plant wxtracts in different industries. Molecules. 27: 8999.

Caetano, A. R. S., M. das G. Cardoso, K. Haddi, G. A. Campolina, B. M. Souza, A. Lunguinho, L. Souza, D. L. Nelson, and J. E. Oliveira. **2022**. *Rosmarinus officinalis* essential oil incorporated into nanoparticles as an efficient insecticide against *Drosophila suzukii* (Diptera: Drosophilidae). Austral Entomol. 61: 265–272.

Calabrese, E. J. 2008. Hormesis: Why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27: 1451.

Calabrese, E. J. 2016. Preconditioning is hormesis part I: Documentation, dose-response features and mechanistic foundations. Pharmacol. Res. 110: 242–264.

Calabrese, E. J., J. J. Giordano, W. J. Kozumbo, R. K. Leak, and T. N. Bhatia. **2018**. Hormesis mediates dose-sensitive shifts in macrophage activation patterns. Pharmacol. Res. 137: 236–249.

Chapman, R. F. 2013. The Insects: Structures and Function. Cambridge University Press, Cambridge.

Cheng, S.-S., H.-T. Chang, C.-Y. Lin, P.-S. Chen, C.-G. Huang, W.-J. Chen, and S.-T. Chang. **2009**. Insecticidal activities of leaf and twig essential oils from *Clausena excavata* against *Aedes aegypti* and *Aedes albopictus* larvae. Pest Manag. Sci. 65: 339–343.

Cheng, S.-S., C.-G. Huang, Y.-J. Chen, J.-J. Yu, W.-J. Chen, and S.-T. Chang. **2009**. Chemical compositions and larvicidal activities of leaf essential oils from two *eucalyptus* species. Bioresour. Technol. 100: 452–456.

Cruz, G. S., V. Wanderley-Teixeira, J. V. Oliveira, F. S. C. Lopes, D. R. S. Barbosa, M. O. Breda, K. A. Dutra, C. A. Guedes, D. M. A. F. Navarro, and A. A. C. Teixeira. **2016**. Sublethal Effects of Essential Oils From *Eucalyptus staigeriana* (Myrtales: Myrtaceae), *Ocimum gratissimum* (Lamiales: Laminaceae), and *Foeniculum vulgare*

(Apiales: Apiaceae) on the Biology of *Spodoptera frugiperda* (Lepidoptera: . J. Econ. Entomol. 109: 660–666.

Cutler, G. C., M. Amichot, G. Benelli, R. N. C. Guedes, Y. Qu, R. R. Rix, F. Ullah, and N. Desneux. **2022**. Hormesis and insects: Effects and interactions in agroecosystems. Sci. Total Environ. 825: 153899.

Deprá, M., J. L. Poppe, H. J. Schmitz, D. C. De Toni, and V. L. S. Valente. **2014**. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest Sci. (2004). 87: 379–383.

Deutsch, C. A., J. J. Tewksbury, M. Tigchelaar, D. S. Battisti, S. C. Merrill, R. B. Huey, and R. L. Naylor. **2018**. Increase in crop losses to insect pests in a warming climate. Science (80-.). 361: 916–919.

van Den Dool, H., and P. Dec. Kratz. **1963**. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A. 11: 463–471.

Echeverría, J., and R. Albuquerque. **2019**. Nanoemulsions of Essential Oils: New Tool for Control of Vector-Borne Diseases and In Vitro Effects on Some Parasitic Agents. Medicines. 6: 42.

EPA Environmental Protection agency. **2022**. Pesticides. Biopesticides. (https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides).

Espinoza, J., C. Medina, W. Aniñir, P. Escobar-Bahamondes, E. Ungerfeld, A. Urzúa, and A. Quiroz. **2021**. Insecticidal, Repellent and Antifeedant Activity of Essential Oils from *Blepharocalyx cruckshanksii* (Hook. & Arn.) Nied. Leaves and *Pilgerodendron uviferum* (D. Don) Florin Heartwood against Horn Flies, *Haematobia irritans* (Diptera. Molecules. 26: 6936.

Etebu, E., and A. B. Nwauzoma. **2014**. A review on sweet orange (*Citrus Sinensis* Osbeck): Health, diseases, and management. Am. J. Res. Commun. 2: 33–70.

Favela-Hernández, J., O. González-Santiago, M. Ramírez-Cabrera, P. Esquivel-Ferriño, and M. Camacho-Corona. **2016**. Chemistry and Pharmacology of *Citrus sinensis*. Molecules. 21: 247.

Fouad, H. A., C. A. G. da Camara, M. M. de Moraes, and J. P. R. de Melo. **2023**. The synergistic effects of five essential oils and eight chiral compounds on deltamethrin-piperonyl butoxide insecticide against *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Asia. Pac. Entomol. 26: 102072.

Gress, B. E., and F. G. Zalom. **2019**. Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. Pest Manag. Sci. 75: 1270–1276.

Guedes, R. N. C., R. R. Rix, and G. C. Cutler. **2022**. Pesticide-induced hormesis in arthropods: Towards biological systems. Curr. Opin. Toxicol. 29: 43–50.

Gupta, V., P. P. Singh, A. Kumar, M. Kumar, T. S. Raghuvanshi, and B. Prakash. **2023**. Nanoencapsulated plant essential oils as a shelf-life enhancer for herbal raw materials, pp. 491–513. *In* Nanotechnol. Herb. Med. Elsevier.

Haddi, K., M. V. Mendes, M. S. Barcellos, J. Lino-Neto, H. L. Freitas, R. N. C. Guedes, and E. E. Oliveira. **2016**. Sexual Success after Stress? Imidacloprid-Induced Hormesis in Males of the Neotropical Stink Bug *Euschistus heros*. PLoS One. 11: e0156616.

Haddi, K., E. E. Oliveira, L. R. A. Faroni, D. C. Guedes, and N. N. S. Miranda. **2015**. Sublethal Exposure to Clove and Cinnamon Essential Oils Induces Hormetic-Like Responses and Disturbs Behavioral and Respiratory Responses in *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 108: 2815–2822.

Haddi, K., L. M. Turchen, L. O. Viteri Jumbo, R. N. Guedes, E. J. Pereira, R. W. Aguiar, and E. E. Oliveira. **2020**. Rethinking biorational insecticides for pest management: unintended effects and consequences. Pest Manag. Sci. 76: 2286–2293.

Hardstone, M. C., X. Huang, L. C. Harrington, and J. G. Scott. **2010**. Differences in Development, Glycogen, and Lipid Content Associated With Cytochrome P450-Mediated Permethrin Resistance in *Culex pipiens quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 47: 188–198.

IRAC. **2006**. Susceptibility test methods series IRAC susceptibility test methods series. 3.

Isman, M. B. 2016. Pesticides Based on Plant Essential Oils: Phytochemical and Practical Considerations, pp. 13–26. *In* .

Isman, M. B. 2020. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. Phytochem. Rev. 19: 235–241.

Isman, M. B., S. Miresmailli, and C. Machial. **2011**. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem. Rev. 10: 197–204.

Jaffar, S., and Y. Lu. **2022**. Toxicity of Some Essential Oils Constituents against Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Insects. 13: 954.

Kelebek, H., and S. Selli. **2011**. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus sinensis* L. Osbeck) orange juice. J. Sci. Food Agric. 91: 1855–1862.

Klauck, V., R. Pazinato, A. Volpato, D. da Silva dos Santos, R. C. V. Santos, M. D. Baldissera, and A. S. da Silva. **2018**. Insecticidal effect of several essential oils against *Musca domestica*. Comp. Clin. Path. 27: 167–172.

Koul, O., S. Walia, and G. S. Dhaliwal. **2008**. Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4: 63–84.

Kumar, P., S. Mishra, A. Malik, and S. Satya. **2012**. Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (*Musca domestica*). Acta Trop. 122: 212–218.

Lima, M. A., F. F. M. Oliveira, G. A. Gomes, P. L. Lavor, G. M. Santiago, and M. G. Nagao-Dias, A. T. Carvalho. **2011**. Evaluation of larvicidal activity of the essential oils of plants species from Brazil against *Aedes aegypti* (Diptera: Culicidae). African J. Biotechnol. 11: 11716-11720.

Lourenço, A. M., K. Haddi, B. M. Ribeiro, R. F. T. Corrêia, H. V. V. Tomé, O. Santos-Amaya, E. J. G. Pereira, R. N. C. Guedes, G. R. Santos, E. E. Oliveira, and R. W. S. Aguiar. **2018**. Essential oil of *Siparuna guianensis* as an alternative tool for improved lepidopteran control and resistance management practices. Sci. Rep. 8: 7215.

Magalhães, A. M., M. Lyege Da Paz, M. Ortiz-Mayo, R. Facanali, A. C. Pinto, and W. Pedro Tadei. **2010**. Chemical Composition and Larvicidal Activity against Aedes aegypti Larvae of Essential Oils from Four Guarea Species. Molecules. 15: 5734–5741.

Matzrafi, M. 2019. Climate change exacerbates pest damage through reduced pesticide efficacy. Pest Manag. Sci. 75: 9–13.

Milind, P., and C. Dev. **2012**. Orange: range of benefits. Int. Res. J. Pharm. 3: 59–63.

Moore, G. A. 2001. Oranges and lemons: clues to the taxonomy of *Citrus* from molecular markers. Trends Genet. 17: 536–540.

Nascimento, A. M. D., T. D. S. Maia, T. E. S. Soares, L. R. A. Menezes, R. Scher, E. V Costa, S. C. H. Cavalcanti, and R. La Corte. **2017**. Repellency and Larvicidal Activity of Essential oils from *Xylopia laevigata*, *Xylopia frutescens*, *Lippia pedunculosa*, and Their Individual Compounds against Aedes aegypti Linnaeus. Neotrop. Entomol. 46: 223–230.

Nation, J. L. 2022. Insect Physiology and Biochemistry. CRC Press, Boca Raton.

O'Bryan, C. A., S. J. Pendleton, P. G. Crandall, and S. C. Ricke. **2015**. Potential of Plant Essential Oils and Their Components in Animal Agriculture – in vitro Studies on Antibacterial Mode of Action. Front. Vet. Sci. 2.

Oliveira, F. M., V. Wanderley-Teixeira, G. S. Cruz, C. T. S. Silva, K. A. Dutra, H. N. Costa, V. A. A. Braga, E. J. Silva, C. A. Guedes, T. J. S. Alves, and Á. A. C. Teixeira. **2021**. Histological, histochemical and energy disorders caused by R-limonene on *Aedes aegypti* L. larvae (Diptera: Culicidae). Acta Trop. 221: 105987.

Palacios, S., A. Bertoni, Y. Rossi, R. Santander, and A. Urzúa. **2009**. Efficacy of Essential Oils from Edible Plants as Insecticides Against the House Fly, *Musca Domestica* L. Molecules. 14: 1938–1947.

Papanastasiou, S. A., E.-M. D. Bali, C. S. Ioannou, D. P. Papachristos, K. D. Zarpas, and N. T. Papadopoulos. **2017**. Toxic and hormetic-like effects of three components of citrus essential oils on adult Mediterranean fruit flies (*Ceratitis capitata*). PLoS One. 12: e0177837.

Pavela, R., K. Kaffkova, and M. Kumšta. **2014**. Chemical composition and larvicidal activity of essential oils from different *Mentha* L. and *Pulegium* species against *Culex quinquefasciatus* say (Diptera: Culicidae). Plant Prot. Sci. 50: 36–42.

Perumalsamy, H., J.-R. Kim, S. M. Oh, J. W. Jung, Y.-J. Ahn, and H. W. Kwon. **2013**. Novel Histopathological and Molecular Effects of Natural Compound Pellitorine on

Larval Midgut Epithelium and Anal Gills of *Aedes aegypti*. PLoS One. 8: e80226.

Pineda, M., E. L. de A. Alves, J. A. Antunes, V. de C. Carvalho, and K. Haddi. **2023**. Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. Agriculture. 13: 404.

Pu, J., Z. Wang, and H. Chung. **2020**. Climate change and the genetics of insecticide resistance. Pest Manag. Sci. 76: 846–852.

Ray, K., M. Mercedes, D. Chan, C. Y. Choi, and J. T. Nishiura. **2009**. Growth and Differentiation of the Larval Mosquito Midgut. J. Insect Sci. 9: 1–13.

Rix, R. R., R. N. C. Guedes, and G. Christopher Cutler. **2022**. Hormesis dose–response contaminant-induced hormesis in animals. Curr. Opin. Toxicol. 30: 100336.

Schlesener, H. D., J. Wollmann, A. P. Krüger, A. M. Nunes, D. Bernardi, and F. R. M. Garcia. **2018**. Biology and fertility life table of *Drosophila suzukii* on artificial diets. Entomol. Exp. Appl.

Shaaya, E., and A. Rafaeli. **2007**. Essential Oils as Biorational Insecticides–Potency and Mode of Action, pp. 249–261. *In* Insectic. Des. Using Adv. Technol. Springer Berlin Heidelberg, Berlin, Heidelberg.

Sharma, S., N. Loach, S. Gupta, and L. Mohan. **2020**. Phyto-nanoemulsion: An emerging nano-insecticidal formulation. Environ. Nanotechnology, Monit. Manag. 14: 100331.

de Souza, L., M. das G. Cardoso, I. F. M. Konig, V. R. F. Ferreira, A. R. S. Caetano, G. A. Campolina, and K. Haddi. **2022b**. Toxicity, Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium verum* Essential Oil in *Drosophila suzukii*. Agriculture. 12: 1667.

Tang, S., J. Liang, C. Xiang, Y. Xiao, X. Wang, J. Wu, G. Li, and R. A. Cheke. **2019**. A general model of hormesis in biological systems and its application to pest management. J. R. Soc. Interface. 16: 20190468.

Tjaden, N. B., C. Caminade, C. Beierkuhnlein, and S. M. Thomas. **2018**. Mosquito-Borne Diseases: Advances in Modelling Climate-Change Impacts. Trends Parasitol. 34: 227–245.

Wangrawa, D. W., E. Ochomo, F. Upshur, N. Zanré, D. Borovsky, C. Lahondere, C. Vinauger, A. Badolo, and A. Sanon. **2022**. Essential oils and their binary combinations have synergistic and antagonistic insecticidal properties against *Anopheles gambiae,* s. l. (Diptera: Culicidae). Biocatal. Agric. Biotechnol. 42: 102347.

Wen, T., M. Sang, M. Wang, L. Han, Z. Gong, X. Tang, X. Long, H. Xiong, and H. Peng. **2021**. Rapid detection of d-limonene emanating from *citrus* infestation by *Bactrocera dorsalis* (Hendel) using a developed gas-sensing system based on QCM sensors coated with ethyl cellulose. Sensors Actuators B Chem. 328: 129048.

Yousof, S. M., R. Atta, I. A. Khalil, M. A. Zayed, and A. Seddek. **2022**. *Citrus sinensis* (Sweet Oranges) Wastes: The Orange Wealth, pp. 261–285. *In* Mediterr. Fruits Bio-Wastes. Springer International Publishing, Cham.

Table 1. Chemical composition of sweet orange *Citrus sinensis* (L.) Osbeck essential oil.

Retention Time (min)	**RI$_{exp}$**	**RI$_{tab}$**	**Constituents**	**Porcentage %**
6.415	934	932	α-Pinene	0.5100
7.747	979	974	β-Pinene	1.2900
8.005	988	988	Myrcene	0.750
9.286	1025	1.020	p-Cymene	0.9400
9495	1030	1024	Limonene	95.29
10543	1058	1.054	ϒ-Terpinene	1.22

Where: RI$_{ex\ pis}$ the experimental retention index and RI$_{tab}$ is the index tabulated in the literature.
In bold are the components considered to be majors.

Table 2. Lethal and sublethal toxicity of orange *C. sinensis* (L.) Osbeck essential oil and its major compound Limonene to adults *Drosophila suzukii*

Compound	**Concentration lethal**	**Concentration lethal µl.mL^{-1}**	**n**	**Fiducial range 95%**	***X*^{2}**	***P***
C. sinensis			1138		12.9724	0.1639
	LC$_1$	0.044		0.034 - 0.055		
	LC$_5$	0.079		0.065 - 0.094		
	LC$_{10}$	0.108		0.092 – 0.125		
	LC$_{20}$	0.159		0.134 – 0.179		
	LC$_{50}$	0.330		0.304 – 0.358		
	LC$_{90}$	1.004		1.004 – 1.148		
(R)-(+)-Limonene			780		4.3821	0.1118
	LC$_1$	7.433		7.278 – 7.544		
	LC$_5$	7.627		7.508- 7.715		
	LC$_{10}$	7.734		7.633- 7.809		
	LC$_{20}$	7.864		7.784 – 7.927		
	LC$_{50}$	8.119		8.064 – 8.175		
	LC$_{90}$	8.524		8.440 – 8.638		

Figures captions

Figure 1. Half-life (LT_{50}) of *Drosophila suzukii* adults exposed to low concentrations (LC_0, control; LC_1; LC_5; LC_{10} and LC_{20}) of orange essential oil *Citrus sinensis* (L.) Osbeck (A-B) and its major component (R)-(+)-Limonene (C-D).

Figure 2. Weight of *Drosophila suzukii* offspring of parents exposed to low concentrations (Control LC_0; LC_1 LC_5 LC_{10} LC_{20}) of orange essential oil *Citrus sinensis* (L.) Osbeck and its major component (R)-(+)-Limonene. **A.** Weight of Pupae; **B.** Weight Females; **C.** Weight Males.

Figure 3. Offspring of *Drosophila suzukii* flies exposed to low concentrations (LC_0, control; LC_1; LC_5; LC_{10}; LC_{20}) of orange essential oil *Citrus sinensis* (L.) Osbeck and its major component (R)-(+)-Limonene. **A-B**. Weekly emergence of females (mean±SE); **C-D.** Weekly emergence of males (mean±SE); **E-F.** Weekly emergenceTotal (Female+male) (mean±SE).

Figure 1

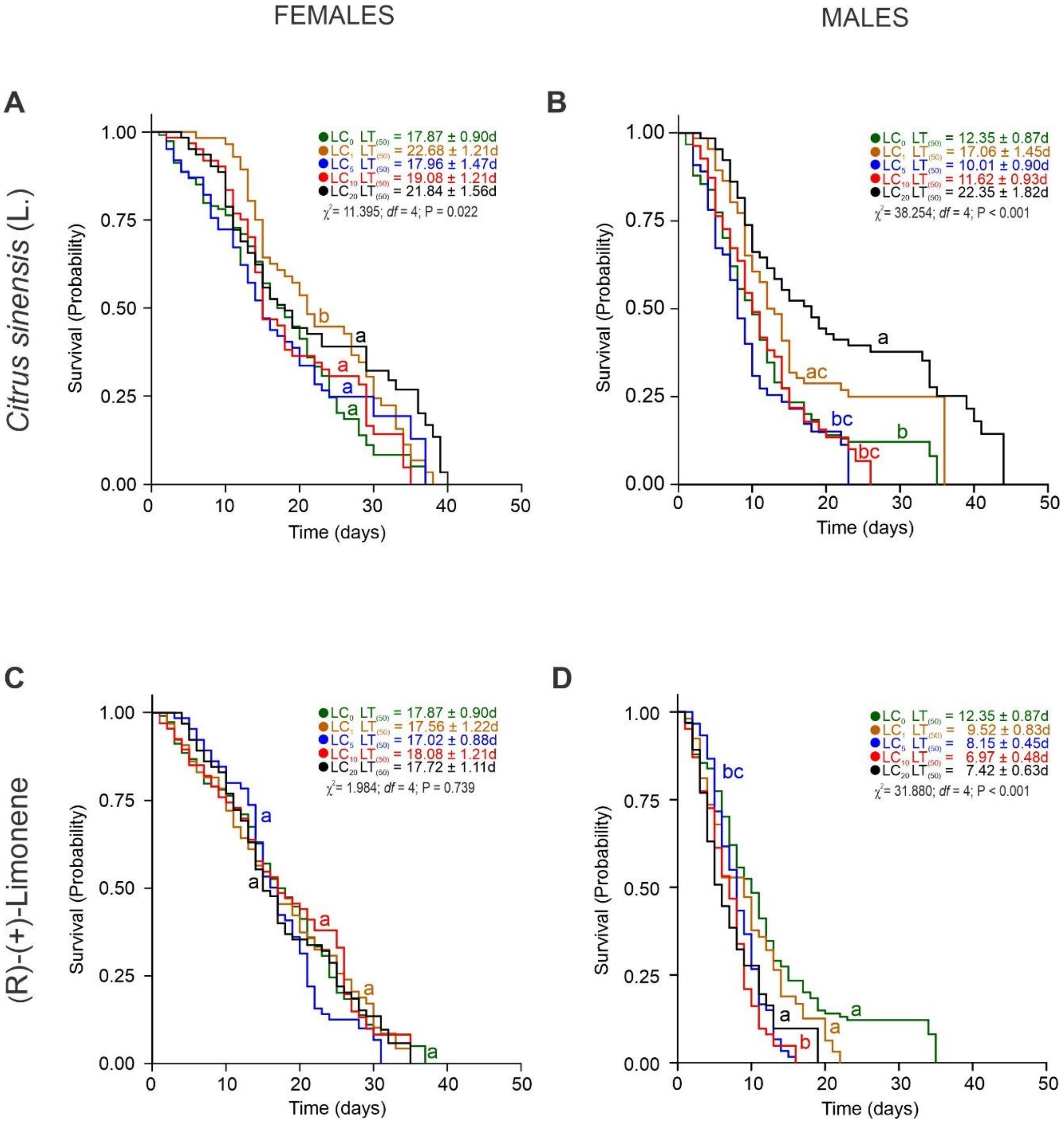

FEMALES
MALES
A
Citrus sinensis (L.)
Survival (Probability)
1.00
0.75
0.50
0.25
0.00
Time (days)
0
10
20
30
40
50
● LC_0 $LT_{(50)}$ = 17.87 ± 0.90d
● LC_1 $LT_{(50)}$ = 22.68 ± 1.21d
● LC_5 $LT_{(50)}$ = 17.96 ± 1.47d
● LC_{10} $LT_{(50)}$ = 19.08 ± 1.21d
● LC_{20} $LT_{(50)}$ = 21.84 ± 1.56d
χ^2= 11.395; df = 4; P = 0.022
b
a
a
a
a
B
● LC_0 $LT_{(50)}$ = 12.35 ± 0.87d
● LC_1 $LT_{(50)}$ = 17.06 ± 1.45d
● LC_5 $LT_{(50)}$ = 10.01 ± 0.90d
● LC_{10} $LT_{(50)}$ = 11.62 ± 0.93d
● LC_{20} $LT_{(50)}$ = 22.35 ± 1.82d
χ^2= 38.254; df = 4; P < 0.001
a
ac
bc
b
bc
C
(R)-(+)-Limonene
● LC_0 $LT_{(50)}$ = 17.87 ± 0.90d
● LC_1 $LT_{(50)}$ = 17.56 ± 1.22d
● LC_5 $LT_{(50)}$ = 17.02 ± 0.88d
● LC_{10} $LT_{(50)}$ = 18.08 ± 1.21d
● LC_{20} $LT_{(50)}$ = 17.72 ± 1.11d
χ^2= 1.984; df = 4; P = 0.739
a
a
a
a
a
D
● LC_0 $LT_{(50)}$ = 12.35 ± 0.87d
● LC_1 $LT_{(50)}$ = 9.52 ± 0.83d
● LC_5 $LT_{(50)}$ = 8.15 ± 0.45d
● LC_{10} $LT_{(50)}$ = 6.97 ± 0.48d
● LC_{20} $LT_{(50)}$ = 7.42 ± 0.63d
χ^2= 31.880; df = 4; P < 0.001
bc
a
a
a
b

Figure 2

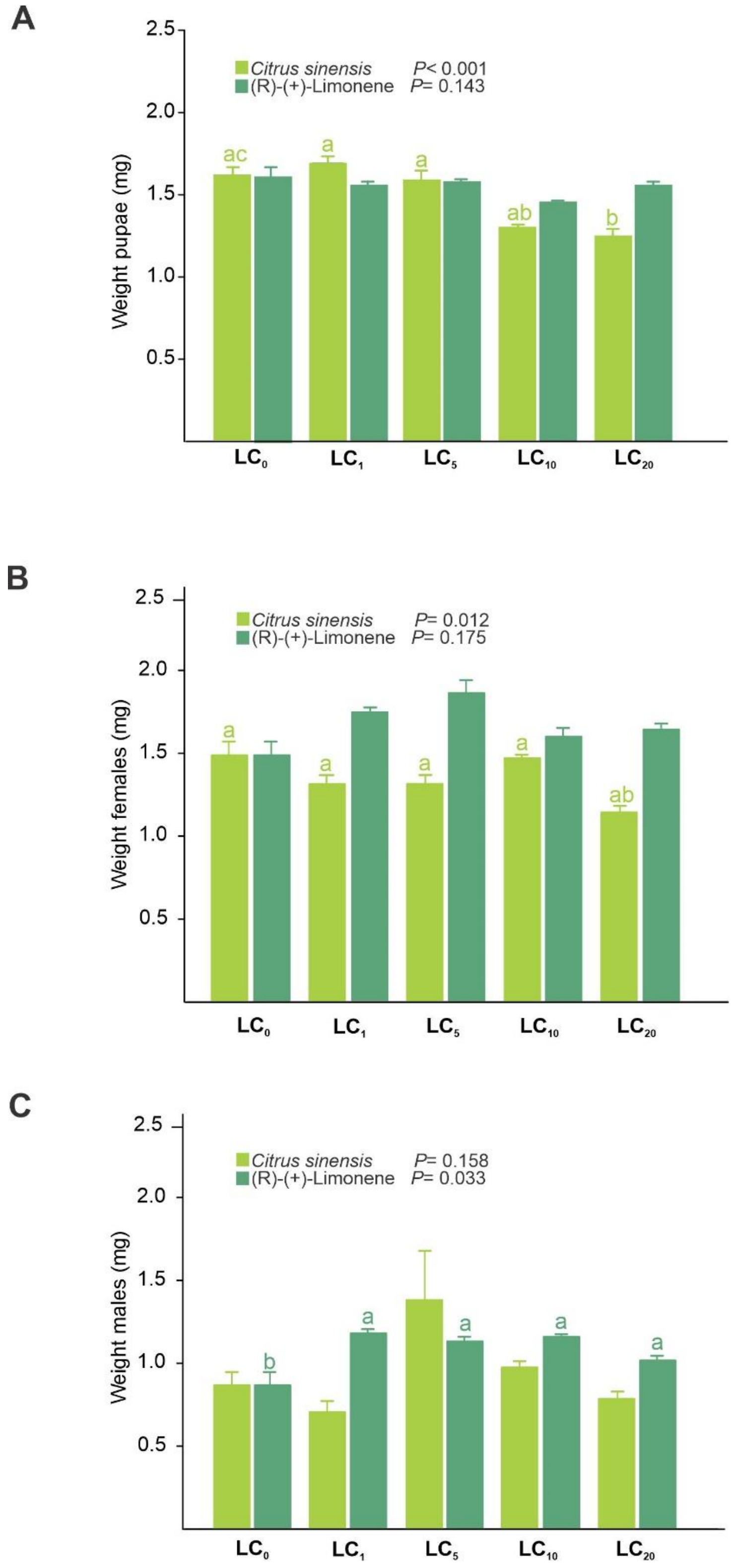
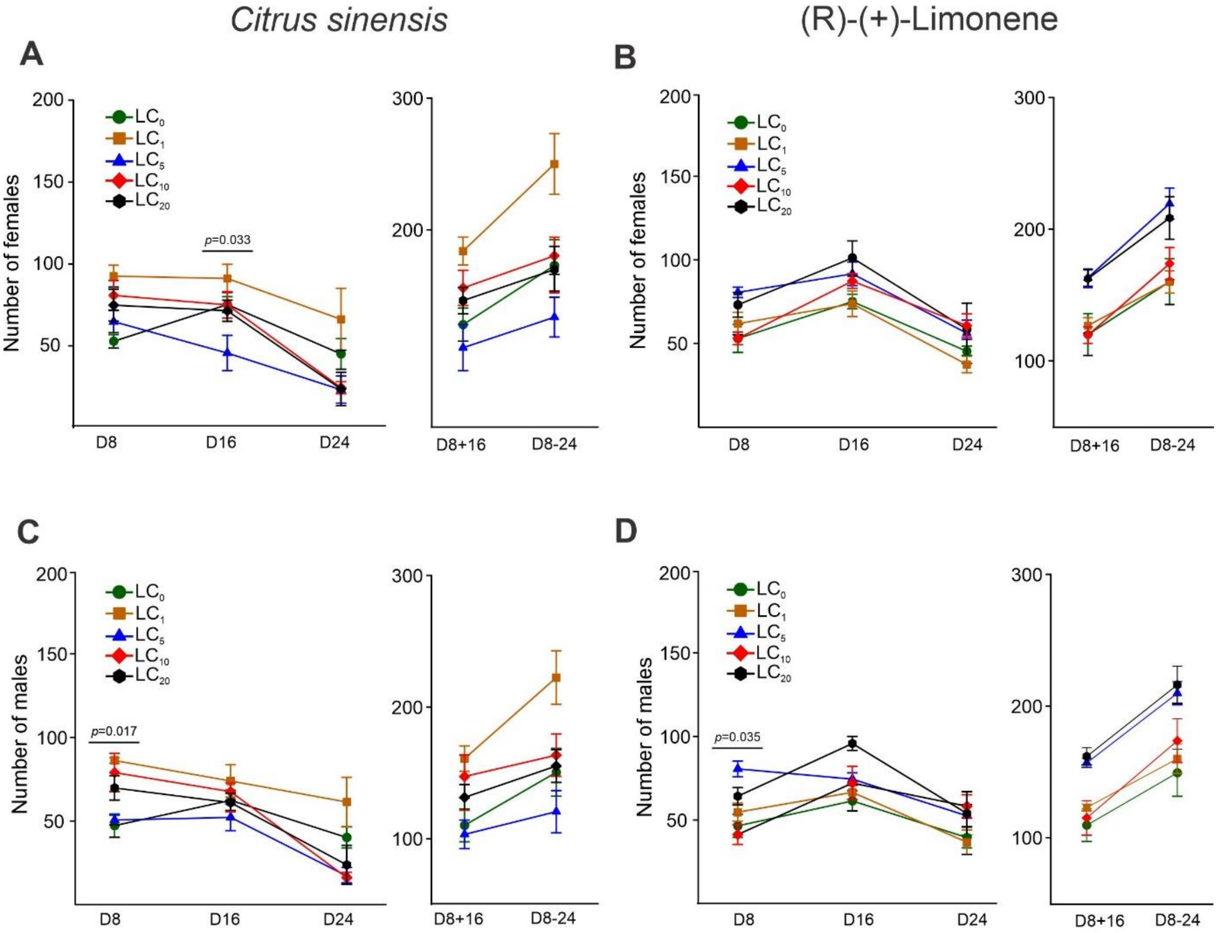
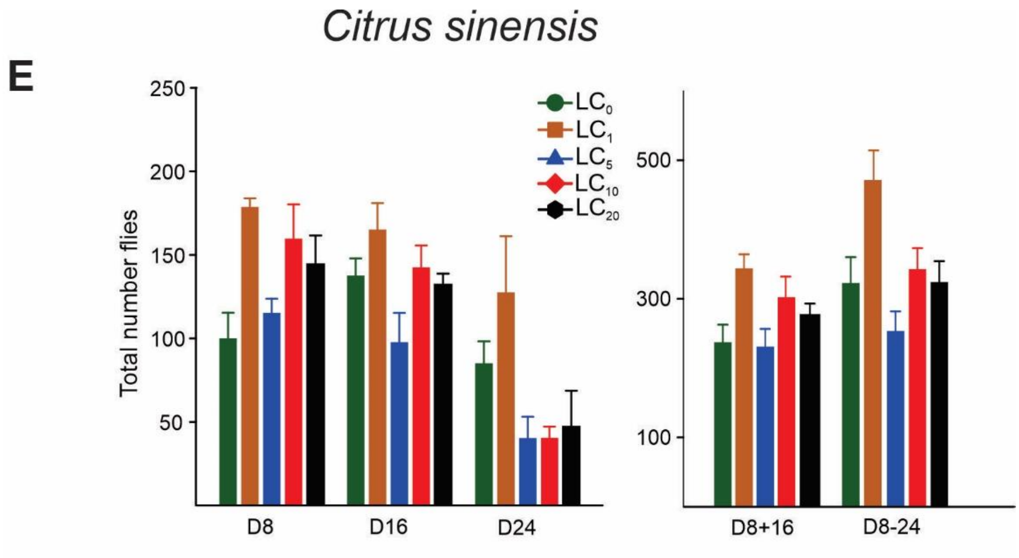
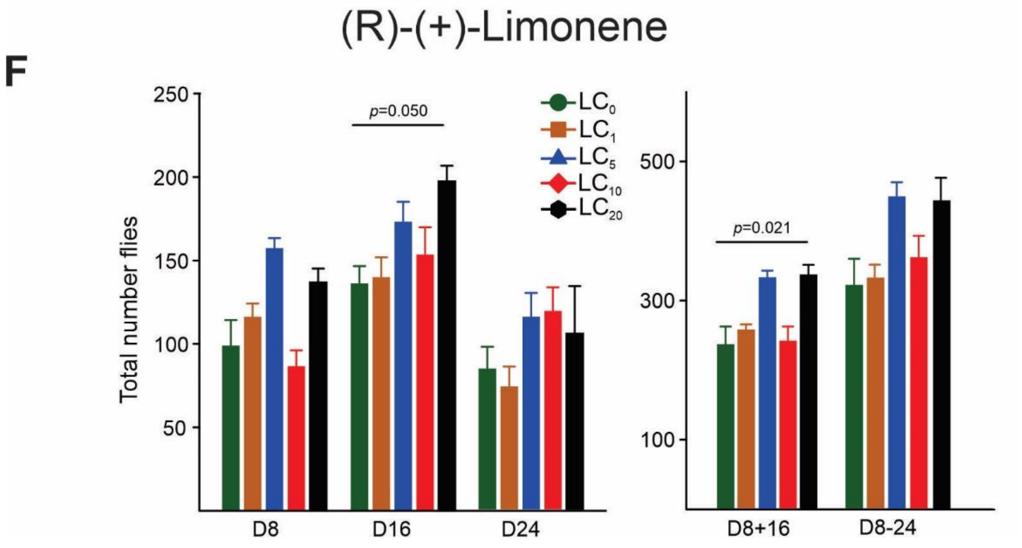

A
2.5
2.0
1.5
1.0
0.5
Weight pupae (mg)
Citrus sinensis P< 0.001
(R)-(+)-Limonene P= 0.143
ac
a
a
ab
b
LC_{0}
LC_{1}
LC_{5}
LC_{10}
LC_{20}
B
2.5
2.0
1.5
1.0
0.5
Weight females (mg)
Citrus sinensis P= 0.012
(R)-(+)-Limonene P= 0.175
a
a
a
a
ab
LC_{0}
LC_{1}
LC_{5}
LC_{10}
LC_{20}
C
2.5
2.0
1.5
1.0
0.5
Weight males (mg)
Citrus sinensis P= 0.158
(R)-(+)-Limonene P= 0.033
b
a
a
a
a
LC_{0}
LC_{1}
LC_{5}
LC_{10}
LC_{20}

Figure 3


Citrus sinensis
(R)-(+)-Limonene
A
B
C
D
LC$_0$
LC$_1$
LC$_5$
LC$_{10}$
LC$_{20}$
Number of females
Number of males
p=0.033
p=0.017
p=0.035
200
150
100
50
300
200
100
D8
D16
D24
D8+16
D8-24


Citrus sinensis
E
Total number flies
250
200
150
100
50
LC_0
LC_1
LC_5
LC_{10}
LC_{20}
D8
D16
D24
500
300
100
D8+16
D8-24


(R)-(+)-Limonene
F
Total number flies
250
200
150
100
50
$p=0.050$
LC_0
LC_1
LC_5
LC_{10}
LC_{20}
D8
D16
D24
500
300
100
$p=0.021$
D8+16
D8-24

ARTICLE IV

Temperature-related biological and morphometric aspects of *Drosophila suzukii* (Matsumura, 1931)

PLoS ONE Journal standards (preliminary version)

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Abstract

Temperature is the most important factor in the life development of insects because it plays a crucial role in their metabolic, physiological, reproductive, and behavioral processes. Exotic pests can adapt to diverse climatic conditions, giving rise to morphotypes, as is the case with *Drosophila suzukii*, the spotted-wing drosophila (SWD) that first arrived in Brazil in 2013. Thus, the present research aimed to determine the effect of two temperatures (20 and 25°C) on the phenotypic plasticity of *D. suzukii*. Adult flies from the *D. suzukii* stock at the Laboratory of Molecular Entomology and Ecotoxicology (MEET-UFLA) were acclimatized, and monitoring was conducted every 5 generations, from F_{10} to F_{30}. We determined the life cycle, body mass, and longevity of adults. Additionally, we quantified morphometric parameters related to phenotypic plasticity: body size (thorax measurements), length, area width, shape, and size of wings, spot area in males, length, and number of ovipositor teeth in females. Survival analyses revealed that at a lower temperature, adult survival is higher. Females reared at 20°C stood out in relation to males, and analysis of variance for linear measurements showed significant differences ($P<0.05$) for all evaluated parameters, except for the number of ovipositor teeth. In males, the area of the spot on the left wing was greater at 20°C. No differences were observed in fluctuating asymmetry analyses; however, multivariate analysis demonstrated differences in wing shape between females and males. We confirmed that temperature effects on phenotypic plasticity, leading to changes in the morphology of the species, giving rise to morphotypes that easily adapt to different environmental conditions, ensuring reproductive success and the invasive capacity of this species.

Keywords: Spotted-wing-drosophila SWD, Temperature, Wings, Ovipositor, Morphometric.

Introduction

In fact, in *Drosophila* flies, the evolution of phenotypic plasticity as a genetic expression has been well-documented in response to different environmental factors [8–11]. Most studies indicate that temperature is the environmental factor that most influences the alteration of physiological processes, reflected in external morphology, enabling them to survive and reproduce successfully [12]. Insects rely on external temperature to control their body temperature [13] and carry out their metabolic and physiological activities [14]. For example, in *Drosophila melanogaster*, genetic analyses demonstrate that certain chromosomal loci are associated with wing shape and size, which are directly affected by temperature changes, thus generating population selection to ensure survival and reproductive success [15–17] Histological and genetic studies show that temperature-induced plasticity varies among sexes, developmental stages, and tissue types, with females exhibiting the most significant morphological changes [18]. However, some authors mention that interspecific variations related to phenotypic plasticity may or may not exist among individuals of the same species [19].

Currently, one of the *Drosophila* species that has gained importance is the Spotted Wing Drosophila (SWD), *D. suzukii*, considered a pest of Asian origin, reported in Brazil ten years ago [20]. The females possess a sclerotized ovipositor capable of piercing healthy fruits to lay eggs; subsequently, the larvae feed on the fruits, causing economic damage through the loss of fruit production [21–23]. This polyphagous pest shows a preference for red fruits, has a broad geographical distribution, and a high adaptability to different environmental conditions. Nevertheless, the optimal temperature for its development is 22.6°C [24]. In recent years, research on SWD has focused on understanding the morphological, biological, ecological, reproductive, and behavioral parameters of this species under different abiotic conditions [25–28]. Among the most studied morphological parameters are the size and shape of the wings, legs, abdomen, and in some cases, the ovipositor [12,29–34]. Therefore, our research aimed to analyze the temperature effect using linear and morphometric measurements, at a transgenerational level, of the main morphological characteristics of male and female *D. suzukii.*

Material and Methods

Insect colony

The flies of D. *suzukii* adults were used in the stock colony of the Laboratory of Molecular Entomology and Eco-Toxicology (MEET) of the Entomology Department at the Federal University of Lavras, (Lavras -MG, Brazil). The flies were maintained in plastic cages (10 cm height × 25 cm Ø) using an artificial diet [35–37] The cages were maintained under controlled conditions of temperature (T: 23 ± 2 ◦C), relative humidity (RH: 60 ± 5%) and scotophase (12H).

Adult acclimatization

Twelve 5-day-old pairs were transferred from the stock colony to a 200 ml glass bottle with 30 ml of artificial diet and covered with foam. Three replicates were placed in biological oxygen demand (BOD) incubators at selected temperatures (15±1 (T_{15}); 20±1 (T_{20}); 25±1 (T_{25}) and 30±1˚C (T_{30})) at 60 ± 10% rh and 12 H of photophase. After 24 hours, the adults were removed and transferred to a new glass bottle and checked daily for longevity. The glass bottle with egg clutches was left in the same conditions until new adults were obtained. Fifteen 5-day-old pairs were transferred back to glass jars with 200 ml of diet. This procedure was performed for each generation to avoid the transposition of generations. The flies were reared and maintained in the BOD until the 30th generation, for the determination of morphological parameters.

Bioassays

Oviposition assay

In a plastic cage (8 cm height × 15 cm Ø) with a window covered with cloth (5 cm x 3 cm) to guarantee the flow of oxygen, 4 Petri dishes of 3 cm Ø were placed with 1 ml of substrate for oviposition (10 g agar; 5 g strawberry gelatin and 425 ml of water) [38], then 15 pairs with 8 days of age of the first generation (F_1) of each temperature (T_{15}; T_{20}; T_{25}; T_{30}) were included. After three hours the adults were removed, except the T_{15} treatment where 60 to 72 hours were necessary to have egg clutches. The presence of eggs was verified with the aid of a stereoscope (zoom 5.0x) Zeiss stemi 2000, each plate was individualized, and the plates were then placed under the same conditions. This procedure was repeated for 4 to 6 generations. Until larval hatching, hourly observations were made to determine the development time (hours) from egg to first instar larvae.

Life cycle

In glass tubes, (8.5 cm high, 2.5 cm diameter) with 1.5 ml of artificial diet and covered with foam, five 8-day-old, first-generation (F_1) were transferred. After 12 hours, the presence of eggs was verified, and the adults were removed. Except for the adults exposed to 15 ^{o}C, which were removed 72 hours later, due to the absence of eggs. The tubes were checked daily with a stereoscope to determine the time (days) of egg-adult development. This procedure was repeated until generation F_6. The experimental design was completely randomized and consisted of 4 replicates for each generation, for each temperature. Each repetition consisted of one tube with the 5 couples as described above.

Adult fly weight

From the F_{10} generation, 10 adults between 6 and hours of emerged, of each sex per replicate, were sacrificed in a lethal chamber (glass tube, cotton on the base covered with white cardboard and impregnated with ethyl acetate.), and subsequently weighed on a Bel Automatic Calibration Analytical Balance M214AI. Flies were labeled and preserved in Eppendorf tubes with 70% alcohol for subsequent morphometric analysis. This procedure was performed 5 times every 5 generations (F_{10} to F_{30}) and was performed only for temperatures of 20°C (T_{20} n=400) and 25°C (T_{25} n=395).

Morphometric measurements

Parameters

To determine the phenotypic plasticity caused by temperature, measurements of the thorax and wings were determined for both sexes, in addition to the ovipositor of females. Adults preserved in alcohol (from the previous stage) were removed and allowed to dry at room temperature for 20 seconds. Then, first, the adults were placed in dorsal thorax dorsal (Txdor) (scutellum+post-scutellum), and lateral thorax right (Txr), thorax left (Txl) (distance between the anterior the pro-pleuron and posterior scutellum) (Figure 1A-B), subsequently in lateral and dorsal position for photography. Then, with the help of dissecting needles the wings of each individual were removed and fixed on glass slides with a mixture of 96% alcohol + glycerin. For the left and right wings of both sexes, three measurements were obtained (Figure 1D-E), length (from the base of the wing to the apical part at the R4+5 vein), width (middle part of the wing, passing through the intersection of the discal-medial and medial-cubital vein) and total area, in males the characteristic spot of this species was also measured (Figure 1E) [30,39]. Finally, in the females, after removal of the wings, the abdomen was placed in a 2 ml Eppendorf tube with 0.5 ml of 10% KOH and placed in a water bath at 70°C for 30 minutes, then, with the help of a brush, all the tissue was removed from the sclerotized valve right to be photographed, the length of the left valva was determined, and the number of teeth in the sclerotized region was counted (Figure 1F).

Photo acquisition

All photographs were obtained using an industrial digital camera UA1000CA (10 megapixel/1/2/3" Aptina CMOS sensor), coupled to a trinocular stereo microscope brand the Zeiss Stemi 2000-C (Germany) microscope has a zoom range of 0.65x -5.0x. Before taking the pictures the camera was always calibrated and placed in the same position, for all the pictures a scale of 1mm was used.

Linear, Morphometry Geometric, and fluctuating asymmetry

Measurements of the thorax, wings, and ovipositor were performed with ImageJ software (l.52q) [40]. Total measurements for the thorax were (n=48) for each sex (female-male) and for each temperature (T_{20}; T_{25}); for the ovipositor (T_{20} n=70; T_{25} n=66). The total number of digitized wings was higher in males (T_{20} n=142; T_{25} n=136) than in females (T_{20} n=96; T_{25} n=96). In order to determine the morphometric alterations in the wings of flies exposed to two temperatures, we first performed a fluctuating asymmetry analysis FA, as recommended by Klingenberg (2015). FA analysis allows for determining if there is sexual dimorphism, if there is symmetry in the wings, and if there are morphological changes caused by stress factors. The right and Left Wings, of males and females were digitized with TPS util64, TPS dig264, and TPS regr64 [41,42]. Fifteen landmarks (for right and left wings) distributed over the entire wing area [43], were defined (Figure 1G) from which X-Y coordinates were extracted and Shape and size information was extracted by a full Procrustes fit [44]. To evaluate measurement error (ME), the protocol proposed by Graham, J.H.; Özener (2016) was followed to ensure the correct procedure for digitization and landmarking. For the case of linear and geometric morphometry, the left wings of the flies were used. An ANOVA was used to determine the differences in the size of the centroid and a Procrustes ANOVA for the Shape, here it was considering the values of individual variation (MS) of the ANOVA. The results were obtained with type 1 error [45–47].

Statistical analysis

The longevity of the parental flies was analyzed with the survival subjected to survival analysis using Kaplan-Meier estimators (Log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). Life cycle, adult weight and linear morphometry measures were subjected to One-way ANOVA and Two-way ANOVA (Generalized linear models, GLM) with Kruskal Wallis, after testing for normality and homoscedasticity assumptions using the Shapiro-Wilk test. Pairwise comparisons were performed by pairwise multiple comparison analysis of means Holm-Sidak method; Tukey's analysis or Dunn's method ($p \leq 0.05$), using the SAS V9 statistical software package (SAS Institute, Cary, NC, USA). All linear measurements (left and right) were determined three times to reduce measurement error. Then, a measurement error analysis was performed to confirm that the changes were due to temperature and to rule out digitization error. The wings were digitized twice, and a Procrustes analysis of variance was performed, comparing the individual values and the mean square (MS) errors of the analysis of variance PROCUSTERS. Subsequently, to determine the variation in wing shape, a principal component analysis (PCA) was performed using the covariance matrix of the symmetric component of the average shape of the population [48]. Subsequently, a canonical variable analysis (CVA) of shape variation was performed to detect differences between temperatures. The relationship between shape and size was analyzed with a multivariate centroid regression, including a confidence ellipse for each group of (Probability: 0.9) with 10.000 interactions. Fluctuating Asymmetry was determined with an ANOVA-Procrusters testing individual × side interactions and individual × side mean squares (MS ind × side). Finally, to determine the significant statistical differences, a multivariate analysis (MANOVA) was performed using temperature as a factor. All statistical analyses and graphs were performed with MorphoJ 2.0 software [48–50]

Results

Parental longevity

The Long-Rank test showed significant statistical differences in the survival time LT_{50} for adults of *D. suzukii* exposed to different temperatures T_{15}, T_{20}, $T_{25,}$ and T_{30} (♀: $\chi^2 = 337.4$; $df = 3$; $p < 0.001$; ♂: $\chi^2 = 8.03$; $df = 3$; $p < 0.001$) (Figure 2). In relation to the mean survival time

LT_{50} for adults of *D. suzukii*, we were able to show that at the maximum temperature, the longevity was lower T_{30} (♀LT_{50}= 8.99 ± 0.20 d; ♂ LT_{50}= 8.03 ± 0.23 d). In contrast, the highest longevity was found in flies exposed to T_{20} (♀LT_{50}= 55.27 ± 1.40 d; ♂ LT_{50}= 54.72 ± 1.49 d) (Figure 2A-B).

Life cycle

The analysis of variance showed significant differences for life cycle length (F=33.590; *df* = 3; *p* <0.001). Development time in days from egg to adult behaved similarly to the longevity of the parental adult flies. As temperature increased the development time in days from egg to adult was shorter T_{30} (11.38 ± 0.20 d), T_{25} (11.45 ± 0.80 d), T_{20} (14.17 ± 0.78 d), and T_{15} (23.88 ± 1.22 d) (Figure 3A; Table 1).

Adult fly weight

In relation to the body mass of adult flies, although a pattern of higher weight was observed in females (T_{20} 2.24 ± 0.03; T_{25} 1.97 ± 0.04 mg) than in males (T_{20} 1.37 ± 0.02; T_{25} 1.28 ± 0.03 mg;), type II ANOVA showed significant differences for the temperature factor (*p* <0.001) and sex (*p* <0.001). There were no differences in body weight at the transgenerational level (Female p = 0.610; male p = 0.770) (Figure 3B) (S Table 1). The flies maintained at 30°C and 15°C did not survive.

Linear Morphometric Analysis

The fluctuating asymmetry analysis showed no differences between the wings of the *D. suzukii* adults analyzed, indicating that the wings are symmetrical, and for this reason only linear measurements of the left wing are presented. Type II ANOVA showed significant differences (P<0.001) for temperature (T_{20}; T_{30}) and sex (female; male) for all linear measurements of length (Figure 4A), width (Figure 4B) and total wing area (Figure 4C), dorsal (Figure 4D) and lateral thorax (Figure 4E) Interestingly, at the transgenerational level, temperature affected thorax measurements more in females (P<0.001) than in males (Tx dorsal: P= 0.712; T left: P= 0.313) (S Table 2). In addition, in females, significant differences were also observed for valve ovipositor size (P<0.001) (Figure 4F), and the number of teeth in each generation (P<0.001) (Figure 4G) (S Table 3). The area of the male characteristic spot also showed statistical differences (P<0.001) when kept at different temperatures. Despite showing some visual differences in spot shape, type II ANOVA showed no significant (P= 0.727) transgenerational differences (Figure 4H; Table 1; S Table 4). However, the transgenerational effect was more evident in all parameters in male wings (Figures 4A, B-H) at both temperatures compared to females (Figure 4; S Table 5).

The value of the mean squares (MS) error of the Procustes ANOVA, after combining two independent measurement datasets of the samples, was lower than the MS value of the individuals, excluding the measurement error of the landmarking process (S Table 6). The principal component analysis (PCA) for total variation in wing shape and size of *D. suzukii* adults maintained at two temperatures (PC1: 26.63; PC2: 10.95; PC3: 9.78; PC4: 9.51 %) showed that the four PCs accounted for 56.88% for females and 54.157% for males (PC1: 22.51; PC2: 11.98; PC3: 10.93; PC4: 8.73 %) (Figures 5A-B, S1). For the factors temperature and transgenerational level of individuals, the Procrustes ANOVA showed significant statistical differences (P<0.001) only for the shape for both sexes (centroid size Females Temperature P= 0.9493; Generation P= 0.9454; Males Temperature P= 0.7376; Generation P= 0.7113) (Table 2).

The plot of the canonical variables to see the differences caused by the effect of temperature showed Mahalanobis distances among groups (P<0.001) (Figures 5C-D). The multivariate regression analysis performed to analyze allometry showed no significant differences (Females P= 0.3962; Males P= 0.6332). Finally, the results of the MANOVA procrustes fluctuating asymmetry caused by temperature, analysis showed significant differences only in shape and not in centroid size (Table 3). Our results identified a small variation between the intersection of landmarks 8-9 (r-m vein) and 10-11 (dm-cu vein). Visually it was more noticeable in females cultured at 20°C compared to males.

Discussion

We found significant effects on the egg-to-adult development time, and median Half-life (LT_{50}) of adults, of *D. suzukii* when they were kept at different constant temperatures. It showed that temperature had a significant effect on the body size and body weight of adult flies. Additionally, morphological changes were evident in both exposed females and males, thus confirming our hypothesis that temperature directly influences the phenotypic plasticity of this species. In fact, we know that insects are ectothermic and heterothermic organisms and their body temperature directly depends on changes in ambient temperature. Thus, the temperature within the optimal range for each species allows each insect species to carry out its metabolism, growth, development, and survival rates normally, making reproduction the fundamental biological activity for maintaining their populations. However, if temperature fluctuations occur, they can have a negative impact on these activities [13,51,52]. We found a negative correlation between temperature and the longevity of adults. At a higher temperature (T_{30}), the average longevity of both female and male adults was 8.5 days under laboratory conditions. Many studies support the findings of this research, confirming that adult flies exhibit greater survival at lower temperatures. In either case, males tend to experience higher mortality rates [38,53,54]. The optimal temperature for the development of *D. suzukii* has been recorded between 22.6 - 25 ^{o}C; however, minimum temperatures between 11.6 -13.2 ^{o}C and maximum temperatures between 30 – 33.6 ^{o}C have also been [24,38,55–58]. Despite these records, our research was unable to achieve more than two generations at the maximum temperature (T_{30}). The few flies that managed to emerge exhibited wing malformations, making it difficult for them to fly and move. Additionally, the flies had a shorter lifespan. It was only possible to observe that they were smaller in size, and the body coloration was lighter compared to flies' growth at other temperatures. The low emergence and survival under conditions (RH 65% T30 ± 2^{o}C) could be attributed to the developmental threshold limit of this species [25]. The low rate of emergence and survival at this temperature indicates that temperatures above 30 °C are close to the upper threshold for the development of the species, as indicated by other studies [56,57] Similarly, as observed in low temperatures (13 °C), where survival rates also decrease, Tonina (et al., 2016) estimated the lower threshold of development under natural conditions (LT = 11.6 °C). Due to technical constraints, it was not possible to obtain more than 4 generations of flies cultured at (T_{15}); however, visually, we observed that they were larger flies, with a darker color, and interestingly, the characteristic wing spot in males was darker. On the other hand, the time and mean temperature of development in insects are directly related to their developmental cycle. In this case, several authors point out that fluctuating temperature and the number of degree days determine each developmental phase (instar) [56,59,60]. Thus, temperature affected the developmental cycle as well as the body size of the insects. At a lower temperature (T_{15}), the egg-to-adult development of *D. suzukii* under laboratory conditions was 23.8 ± 1.22 days, while at a higher temperature, it was significantly shorter at 11.45 ± 0.80 days. Similarly, there was an effect on the change in body size of females and males. The differences became more evident after 30 generations, with females being larger and having greater weight compared to males. Thus, flies maintained at 20ºC surpassed those kept at 25ºC. Temperature directly influences the growth rate of *Drosophila* because its physiological processes are regulated by biochemical and molecular mechanisms. Therefore, temperature alterations can affect the insect's reproduction due to thermal stress that directly impacts its endocrine system [61]. For example, in the last larval instar of *Drosophila melanogaster*, the secretion of juvenile hormones stops upon reaching the pupal stage, and this is directly reflected in the body size of the flies [62,63]. The temperatures to which the flies were exposed affect cell size, and studies have shown that smaller cells are more tolerant to acute temperature fluctuations as they can supply more oxygen. On the other hand, larger cells are more tolerant to chronic thermal stress [64,65]. The cell size in fruit flies is directly reflected in body size, and therefore, it is reflected in morphological changes that allow them to adapt to the new conditions to which they are exposed [66,67]. Among the most commonly used methods to analyze the phenotypic plasticity of a species is geometric and linear morphometrics, which, in this case, allowed us to determine the effect of temperature on variations in the shape and size of the thorax, wings, and ovipositor in *D. suzukii* flies. Our experiments showed no difference in thorax measurements at the transgenerational level. However, after 30 generations in adults exposed to constant temperatures, the area and length of the thorax were significantly larger, especially in females exposed to T_{20} compared to males and females exposed to T_{25}. Other linear measurements were related to thorax size, with lower temperatures tending to increase thorax size, proportionally leading females to have larger wings compared to males, and with an increase in temperature. Stockton et al (2020) found similar results, where the morphology and survival of *D. suzukii* adults are higher at lower temperatures, as well as the melanization of the abdomen in adults [39]. So, temperature is an environmental factor that is directly involved in the adaptation and survival capacity of this species. It is established that *D. suzukii* adults survive cold periods and easily adapt when they have the right conditions for reproduction. However, this phenotypic plasticity reflected in the change in body color and size comes at a cost to their reproduction. It has been recorded that they employ the reproductive strategy of diapause. Nevertheless, it is not entirely clear whether this is due to changes in temperature, photoperiod, humidity, type of food, or external morphology [15,28,29,68]. In the case of females, the sclerotized length measurement was greater in those cultivated at T_{20} degrees compared to T_{25}, and conversely, the number of teeth showed no statistical differences. Visually, it was observed that the teeth at the higher temperature were thinner and more delicate, possibly representing an adaptation of females, as fruits from warmer climates tend to have a thinner exocarp [69]. Indeed, the reproductive success and the significance of this species lie in the fact that females have a serrated and sclerotized ovipositor, allowing them to lay eggs inside the fruit, favoring their invasive success. Other research suggests that at higher temperatures, there are small changes in the shape and size of the ovipositor, indicating a strong adaptation of this organ that plays a mechanical role concerning [30,32]. On the contrary, several authors also emphasize that diet or host type does not cause changes in the morphology of the [70,71]. Multiple research studies point to a relationship between temperature and the phenotypic plasticity of this species. However, it is crucial to consider that these are experiments with flies cultivated in controlled conditions, and one must account for the broad geographical distribution of this species, which exhibits morphological and behavioral differences [72].The only study that explored the number and recorded significant differences in ovipositor size and the number of teeth was a research project that compared three strains with different geographic origins (France, the United States, and Japan). Conversely, they did not register differences in terms of body and wing size [73]. The wings showed differences in width and length, which were more pronounced in females compared to males. It has been confirmed through various methods that developmental temperature has a strong effect on wing size, and flies raised at lower temperatures exhibit changes in wing size compared to flies raised at other temperatures. Our geometric morphometric results on the wings demonstrated that there are two morphotypes in wing shape, following a comparison of the average shape in the wings of female and male flies exposed to 20 and 25°C. The pattern of veins in *Drosophila* is highly characteristic; however, with the superimposition of points, a slight difference can be observed in the transverse veins r-m and dm-cu. For several authors, specific morphotypes, as in this case, caused by temperature, can affect the flight performance and behavior of various *Drosophila* species [74]. In *D. suzukii*, it has already been documented that temperature, in addition to affecting morphology, directly influences flight parameters such as speed and acceleration [31,38,72,75]. Although our results did not show differences in fluctuating asymmetry in both sexes, interestingly in males, the wing spot area was asymmetrical. The left-wing spot in males cultivated at 20^{o}C was larger compared to the right-wing spot, and its color was darker compared to the spots of males at 25°C. The only study indicating similar results to our research is that of Ceferino, Varón et al (2020), who also detected consistent directional asymmetry in spot size, favoring the right side, indicating a lateralized sexual behavior in all populations and temperatures. Thus, temperature directly affects reproduction and the phenotypic plasticity of

D. suzukii, giving rise to morphotypes that easily adapt to environmental conditions. This has undoubtedly been the key to its geographical dispersion and classification as an invasive exotic pest.

Conclusion

Based on our results, we can conclude that temperature is a stressor for spotted-wing drosophila. We found variations between temperatures 20 and 25^{o}C, which directly influenced the development time from egg to adult. Similarly, differences were observed between morphometric parameters (thorax, wings, and ovipositor). Phenotypic plasticity was more notable in males' wings, which showed more visual phenotypic characters than females. This suggests that the phenotypic plasticity of *D. suzukii* allows it to adapt easily to temperature changes, ensuring its reproductive success.

Supporting information

Word document: Supplementary tables
Supplemental Image 1 in PDF

Acknowledgments

This research was funded by the National Council of Scientific and Technological Development (CNPq), Minas Gerais State Foundation for Research Aid (FAPEMIG, APQ-02230-21), and Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Capes, Brazil) (Finance code 001).

Author Contributions

Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources: MP, KH., Methodology, Writting-Original draft: MP, KH., Project administration**,** Supervision, Validation, Visualization, Writing-review & editing**:** KH

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.

References

1. Pigliucci M. Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol. 2005;20: 481–486. doi:10.1016/j.tree.2005.06.001

2. Lin X, Xu Y, Jiang J, Lavine M, Lavine LC. Host quality induces phenotypic plasticity in a wing polyphenic insect. Proc Natl Acad Sci. 2018;115: 7563–7568. doi:10.1073/pnas.1721473115

3. Zhang C-X, Brisson JA, Xu H-J. Molecular Mechanisms of Wing Polymorphism in Insects. Annu Rev Entomol. 2019;64: 297–314. doi:10.1146/annurev-ento-011118-112448

4. Brisson JA. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. Philos Trans R Soc B Biol Sci. 2010;365: 605–616. doi:10.1098/rstb.2009.0255

5. West-Eberhard MJ. Developmental plasticity and the origin of species differences. Proc Natl Acad Sci. 2005;102: 6543–6549. doi:10.1073/pnas.0501844102

6. Moczek AP. Phenotypic plasticity and diversity in insects. Philos Trans R Soc B Biol Sci. 2010;365: 593–603. doi:10.1098/rstb.2009.0263

7. Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol. 2010;25: 459–467. doi:10.1016/j.tree.2010.05.006

8. Levine MT, Eckert ML, Begun DJ. Whole-Genome Expression Plasticity across Tropical and Temperate *Drosophila melanogaster* Populations from Eastern Australia. Mol Biol Evol. 2011;28: 249–256. doi:10.1093/molbev/msq197

9. Yampolsky LY, Glazk G V., Fry JD. Evolution of gene expression and expression plasticity in long-term experimental populations of Drosophila melanogaster maintained under constant and variable ethanol stress. Mol Ecol. 2012;21: 4287–4299. doi:10.1111/j.1365-294X.2012.05697.x

10. Chen J, Nolte V, Schlötterer C. Temperature Stress Mediates Decanalization and Dominance of Gene Expression in *Drosophila melanogaster*. Gibson G, editor. PLOS

Genet. 2015;11: e1004883. doi:10.1371/journal.pgen.1004883

11. Huang Y, Agrawal AF. Experimental Evolution of Gene Expression and Plasticity in Alternative Selective Regimes. Begun DJ, editor. PLOS Genet. 2016;12: e1006336. doi:10.1371/journal.pgen.1006336

12. Mallard F, Nolte V, Schlötterer C. The Evolution of Phenotypic Plasticity in Response to Temperature Stress. George Z, editor. Genome Biol Evol. 2020;12: 2429–2440. doi:10.1093/gbe/evaa206

13. González-Tokman D, Córdoba-Aguilar A, Dáttilo W, Lira-Noriega A, Sánchez-Guillén RA, Villalobos F. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. Biol Rev. 2020;95: 802–821. doi:10.1111/brv.12588

14. Culos GJ, Tyson RC. Response of poikilotherms to thermal aspects of climate change. Ecol Complex. 2014;20: 293–306. doi:10.1016/j.ecocom.2014.09.003

15. Hoffmann AA, Sgrò CM. Climate change and evolutionary adaptation. Nature. 2011;470: 479–485. doi:10.1038/nature09670

16. Hoffmann AA, Sørensen JG, Loeschcke V. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J Therm Biol. 2003;28: 175–216. doi:10.1016/S0306-4565(02)00057-8

17. Rako L, Hoffmann AA. Complexity of the cold acclimation response in *Drosophila melanogaster*. J Insect Physiol. 2006;52: 94–104. doi:10.1016/j.jinsphys.2005.09.007

18. Voigt S, Kost L. Differences in temperature-sensitive expression of PcG-regulated genes among natural populations of *Drosophila melanogaster*. Lott SE, editor. G3 Genes|Genomes|Genetics. 2021;11. doi:10.1093/g3journal/jkab237

19. Kimura MT. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia. 2004;140: 442–449. doi:10.1007/s00442-004-1605-4

20. Deprá M, Poppe JL, Schmitz HJ, De Toni DC, Valente VLS. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J Pest Sci (2004). 2014;87: 379–383. doi:10.1007/s10340-014-0591-5

21. Gress BE, Zalom FG. Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. Pest Manag Sci. 2019;75: 1270–1276. doi:10.1002/ps.5240

22. Schlesener HD, Wollmann J, Krüger AP, Nunes AM, Bernardi D, Garcia FRM. Biology and fertility life table of *Drosophila suzukii* on artificial diets. Entomol Exp Appl. 2018. doi:10.1111/eea.12736

23. Andreazza F, Haddi K, Oliveira EE, Ferreira JAM. *Drosophila suzukii* (Diptera: Drosophilidae) Arrives at Minas Gerais State, a Main Strawberry Production Region in Brazil. Florida Entomol. 2016;99: 796–798. doi:10.1653/024.099.0439

24. Winkler A, Jung J, Kleinhenz B, Racca P. Estimating temperature effects on *Drosophila suzukii,* life cycle parameters. Agric For Entomol. 2021;23: 361–377. doi:10.1111/afe.12438

25. Stockton D, Wallingford A, Rendon D, Fanning P, Green CK, Diepenbrock L, et al. Interactions Between Biotic and Abiotic Factors Affect Survival in Overwintering *Drosophila suzukii* (Diptera: Drosophilidae). Environ Entomol. 2019;48: 454–464. doi:10.1093/ee/nvy192

26. Grassi A, Gottardello A, Dalton DT, Tait G, Rendon D, Ioriatti C, et al. Seasonal Reproductive Biology of *Drosophila suzukii* (Diptera: Drosophilidae) in Temperate Climates. Environ Entomol. 2018;47: 166–174. doi:10.1093/ee/nvx195

27. Toxopeus J, Jakobs R, Ferguson L V., Gariepy TD, Sinclair BJ. Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. J Insect Physiol. 2016;89: 37–51. doi:10.1016/j.jinsphys.2016.03.006

28. Leach H, Stone J, Van Timmeren S, Isaacs R. Stage-Specific and Seasonal Induction of the Overwintering Morph of Spotted Wing Drosophila (Diptera: Drosophilidae). J Insect Sci. 2019;19. doi:10.1093/jisesa/iez067

29. Tran AK, Hutchison WD, Asplen MK. Morphometric criteria to differentiate *Drosophila suzukii* (Diptera: Drosophilidae) seasonal morphs. Rossi S, editor. PLoS One. 2020;15: e0228780. doi:10.1371/journal.pone.0228780

30. Varón-González C, Fraimout A, Delapré A, Debat V, Cornette R. Limited thermal

plasticity and geographical divergence in the ovipositor of *Drosophila suzukii*. R Soc Open Sci. 2020;7: 191577. doi:10.1098/rsos.191577

31. Fraimout A, Jacquemart P, Villarroel B, Aponte DJ, Decamps T, Herrel A, et al. Phenotypic plasticity of *Drosophila suzukii* wing to developmental temperature: implications for flight. J Exp Biol. 2018;221: jeb166868. doi:10.1242/jeb.166868

32. Clemente M, Fusco G, Tonina L, Giomi F. Temperature-induced phenotypic plasticity in the ovipositor of the invasive species *Drosophila suzukii*. J Therm Biol. 2018;75: 62–68. doi:10.1016/j.jtherbio.2018.05.001

33. Pajač Živković I, Lemic D, Mešić A, Barić B, Órdenes R, Benítez HA. Effect of fruit host on wing morphology in Drosophila suzukii (Diptera: Drosophilidae): A first view using geometric morphometrics. Entomol Res. 2018;48: 262–268. doi:10.1111/1748-5967.12278

34. Jardeleza MG, Koch JB, Pearse IS, Ghalambor CK, Hufbauer RA. The roles of phenotypic plasticity and adaptation in morphology and performance of an invasive species in a novel environment. Ecol Entomol. 2022;47: 25–37. doi:10.1111/een.13087

35. Andreazza F, Bernardi D, Marangon RB, Botton M, Nava DE. Técnica de criação de *Drosophila suzukii* (Matsumura, 1931)(Diptera: Drosophilidae) em dieta artificial. Embrapa Clima Temperado-Boletim Pesqui e Desenvolv (INFOTECA-E). 2016; 23.

36. Mendonca L de P, Oliveira EE, Andreazza F, Rezende SM, Faroni LRD, Guedes RNC, et al. Host Potential and Adaptive Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Barbados Cherries. J Econ Entomol. 2019. doi:10.1093/jee/toz195

37. Pineda M, Alves EL de A, Antunes JA, Carvalho V de C, Haddi K. Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. Agriculture. 2023;13: 404. doi:10.3390/agriculture13020404

38. Schlesener DCH, Wollmann J, Krüger AP, Martins LN, Teixeira CM, Bernardi D, et al. Effect of temperature on reproduction, development, and phenotypic plasticity of *Drosophila suzukii* in Brazil. Entomol Exp Appl. 2020;168: 817–826. doi:10.1111/eea.12954

39. Stockton DG, Loeb GM. Winter warm-up frequency and the degree of temperature fluctuations affect survival outcomes of spotted-wing drosophila winter morphotypes. J Insect Physiol. 2021;131: 104246. doi:10.1016/j.jinsphys.2021.104246

40. Ferreira T, Rasband WS. ImageJ. In: ImageJ User Guide. 2023 p. imagej.nih.gov/ij/docs/guide/.

41. Rohlf F. TPSdig, v. 2.17. Digitize Landmarks and Outlines. Department of Ecology and Evolution, State University at Stony Brook. New York, NY, USA,: New York, NY, USA,; 2013.

42. F.J. R. tpsUTIL. Version 1.28. New York: Department of Ecology and Evolution, State University of New York at Stony Brook; 2004.

43. Bookstein FL. Morphometric Tools for Landmark Data. Cambridge, UK: Cambridge University Press; 1997.

44. Rohlf FJ, Slice D. Extensions of the Procrustes Method for the Optimal Superimposition of Landmarks. Syst Zool. 1990;39: 40. doi:10.2307/2992207

45. Graham J, Özener B. Fluctuating Asymmetry of Human Populations: A Review. Symmetry (Basel). 2016;8: 154. doi:10.3390/sym8120154

46. Benítez H, Lemic D, Villalobos-Leiva A, Bažok R, Órdenes-Claveria R, Pajač Živković I, et al. Breaking Symmetry: Fluctuating Asymmetry and Geometric Morphometrics as Tools for Evaluating Developmental Instability under Diverse Agroecosystems. Symmetry (Basel). 2020;12: 1789. doi:10.3390/sym12111789

47. Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, et al. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Manag Sci. 2017;73: 1883–1899. doi:10.1002/ps.4554

48. Klingenberg CP, Barluenga M, Meyer A. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution (N Y). 2002;56: 1909–1920. doi:10.1111/j.0014-3820.2002.tb00117.x

49. Klingenberg CP. Morpho J: an integrated software package for geometric morphometrics. Mol Ecol Resour. 2011;11: 353–357. doi:10.1111/j.1755-

0998.2010.02924.x

50. Klingenberg C. Analyzing Fluctuating Asymmetry with Geometric Morphometrics: Concepts, Methods, and Applications. Symmhmnetry (Basel). 2015;7: 843–934. doi:10.3390/sym7020843

51. Nedvěd O. Temperature, Effects on Development and Growth. Encyclopedia of Insects. Elsevier; 2009. pp. 990–993. doi:10.1016/B978-0-12-374144-8.00261-7

52. Régnière J, Powell J, Bentz B, Nealis V. Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. J Insect Physiol. 2012;58: 634–647. doi:10.1016/j.jinsphys.2012.01.010

53. Shope J, Polk D, Mansue C, Rodriguez-Saona C. The contrasting role of climate variation on the population dynamics of a native and an invasive insect pest. Garganese F, editor. PLoS One. 2023;18: e0284600. doi:10.1371/journal.pone.0284600

54. Hamby KA, E. Bellamy D, Chiu JC, Lee JC, Walton VM, Wiman NG, et al. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. J Pest Sci (2004). 2016;89: 605–619. doi:10.1007/s10340-016-0756-5

55. Kinjo H, Kunimi Y, Nakai M. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). Appl Entomol Zool. 2014;49: 297–304. doi:10.1007/s13355-014-0249-z

56. Tochen S, Dalton DT, Wiman N, Hamm C, Shearer PW, Walton VM. Temperature-Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. Environ Entomol. 2014;43: 501–510. doi:10.1603/EN13200

57. Ryan GD, Emiljanowicz L, Wilkinson F, Kornya M, Newman JA. Thermal Tolerances of the Spotted-Wing *Drosophila Drosophila suzukii* (Diptera: Drosophilidae). J Econ Entomol. 2016;109: 746–752. doi:10.1093/jee/tow006

58. Enriquez T, Colinet H. Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. PeerJ. 2017;5: e3112. doi:10.7717/peerj.3112

59. Bristow L V., Grundel R, Dzurisin JDK, Wu GC, Li Y, Hildreth A, et al. Warming

experiments test the temperature sensitivity of an endangered butterfly across life history stages. J Insect Conserv. 2023. doi:10.1007/s10841-023-00518-3

60. Tonina L, Mori N, Giomi F, Battisti A. Development of *Drosophila suzukii* at low temperatures in mountain areas. J Pest Sci (2004). 2016;89: 667–678. doi:10.1007/s10340-016-0730-2

61. Du Y, Ma CS, Zhao QH, Ma G, Yang HP. Effects of heat stress on physiological and biochemical mechanisms of insects: A literature review. acta ecol. 2007;27: 1565–1572.

62. Mirth CK, Tang HY, Makohon-Moore SC, Salhadar S, Gokhale RH, Warner RD, et al. Juvenile hormone regulates body size and perturbs insulin signaling in *Drosophila*. Proc Natl Acad Sci. 2014;111: 7018–7023. doi:10.1073/pnas.1313058111

63. Zhang X, Li S, Liu S. Juvenile Hormone Studies in *Drosophila melanogaster*. Front Physiol. 2022;12. doi:10.3389/fphys.2021.785320

64. Verspagen N, Leiva FP, Janssen IM, Verberk WCEP. Effects of developmental plasticity on heat tolerance may be mediated by changes in cell size in *Drosophila melanogaster*. Insect Sci. 2020;27: 1244–1256. doi:10.1111/1744-7917.12742

65. Panel ADC, Pen I, Pannebakker BA, Helsen HHM, Wertheim B. Seasonal morphotypes of *Drosophila suzukii* differ in key life-history traits during and after a prolonged period of cold exposure. Ecol Evol. 2020;10: 9085–9099. doi:10.1002/ece3.6517

66. Leiva FP, Boerrigter JGJ, Verberk WCEP. The role of cell size in shaping responses to oxygen and temperature in fruit flies. Funct Ecol. 2023;37: 1269–1279. doi:10.1111/1365-2435.14294

67. Czarnoleski M, Dragosz-Kluska D, Angilletta MJ. Flies developed smaller cells when temperature fluctuated more frequently. J Therm Biol. 2015;54: 106–110. doi:10.1016/j.jtherbio.2014.09.010

68. Shearer PW, West JD, Walton VM, Brown PH, Svetec N, Chiu JC. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC Ecol. 2016;16: 11. doi:10.1186/s12898-016-0070-3

69. Atallah J, Teixeira L, Salazar R, Zaragoza G, Kopp A. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proc

R Soc B Biol Sci. 2014;281: 20132840. doi:10.1098/rspb.2013.2840

70. Green JE, Cavey M, Médina Caturegli E, Aigouy B, Gompel N, Prud'homme B. Evolution of Ovipositor Length in *Drosophila suzukii* Is Driven by Enhanced Cell Size Expansion and Anisotropic Tissue Reorganization. Curr Biol. 2019;29: 2075-2082.e6. doi:10.1016/j.cub.2019.05.020

71. Rendon D, Buser J, Tait G, Lee JC, Walton VM. Survival and Fecundity Parameters of Two *Drosophila suzukii* (Diptera: Drosophilidae) Morphs on Variable Diet Under Suboptimal Temperatures. J Insect Sci. 2018;18. doi:10.1093/jisesa/iey113

72. Varón-González C, Fraimout A, Debat V. *Drosophila suzukii* wing spot size is robust to developmental temperature. Ecol Evol. 2020;10: 3178–3188. doi:10.1002/ece3.5902

73. Akutsu J, Matsuo T. Intra-species variation of ovipositor morphology and oviposition preference in *Drosophila suzukii* (Diptera: Drosophilidae). Appl Entomol Zool. 2023;58: 323–328. doi:10.1007/s13355-023-00835-8

74. Gilchrist GW. Plastic and Genetic Variation in Wing Loading as a Function of Temperature Within and Among Parallel Clines in *Drosophila subobscura*. Integr Comp Biol. 2004;44: 461–470. doi:10.1093/icb/44.6.461

75. Pajač Živković I, Lemic D, Mešić A, Barić B, Órdenes R, Benítez HA. Effect of fruit host on wing morphology in *Drosophila suzukii* (Diptera: Drosophilidae): A first view using geometric morphometrics. Entomol Res. 2018;48: 262–268. doi:10.1111/1748-5967.12278

Table 1. Duration of developmental stages of *Drosophila suzukii* at different Temperatures Means and standard deviations (μ ± SE) of Performance Scores.

			Development time (days)				
Temperature °C	**Generations**	**n**	**Egg**	**Larva-prepupa**	**Pre-pupa**	**Pupa-adult**	**Egg - adult**
15	4	4	3.00 ±0.38	9.31 ± 0.85	2.62 ± 0.46	8.93 ± 0.46	23.87 ± 1.12^{a}
20	6	4	0.50 ± 0.00	7.42 ± 0.21	1.00 ± 0.00	5.25 ± 0.00	14.17 ± 0.21^{b}
25	6	4	0.50 ± 0.00	5.25 ± 0.62	1.00 ± 0.00	4.70 ± 0.53	11.46 ± 0.80^{c}
30	2	3	0.45± 0.01	5.50 ± 0.50	0.93 ± 0.08	4.50 ± 0.33	11.38 ± 0.19^{c}

Test Multiple Comparison of means of Dunn's Method, different letters, with statistically significant difference (P = <0.001)

Table 2. Procrustes ANOVA of *Drosophila suzukii* adults to determine the effect of temperature on female and male wing size and shape for matched symmetry. Degrees of freedom (df). F-statistics and parametric P-values for each effect in females and males.

Effect	df	F	P
Females			
Centroid size			
Temperature	1	0.0	0.9493
Generation	3	0.12	0.9454
Individual	80	0.93	0.6314
Side	1	0.0	0.9721
Shape			
Temperature	26	27.05	<0.0001
Generation	78	1.82	<0.0001
Individual	2080	2.52	<0.0001
Side	26	1.32	0.3500
Males			
Centroid size			
Tempreratura	1	0.11	0.7376
Generation	4	2.48	0.7113
Individual	121	0.95	0.6224
Side	1	0.14	0.7052
Shape			
Temperature	26	34.44	<0.0001
Generation	104	2.03	<0.0001
Individual	3146	3.313	<0.0001
Side	26	1.11	0.3206

Table 3 Procrustes ANOVA of *Drosophila suzukii* adults to determine the effect of temperature on female and male wing size and shape for Fluctuating asymmetry. Degrees of freedom (df). F-statistics and parametric P-values for each effect in females and males.

Effect	df	F	P	Pillai tr*	P (param.)
Females					
Centroid size					
Temperature	1	0	0.9740		
Generation	3	0.16	0.9225		
Individual	83	0.93	0.6290		
Side	1	0	0.9.721		
Individual*side	86	1.01	0.4671		
Error	174				
Shape	Temperature				
Temperature	26	26.05	<0.0001	0.94	<0.0001
Generation	78	2.00	<0.0001	0.0060	0.0060
Individual	2158	3.63	<0.0001	16.96	<0.0001
Side	26	1.11	0.3173	0.31	0.4415
Individual*side	2236	2.54	<0.0001	13.27	<0.0001
Error	4524				
Males					
Centroid size					
Tempreratura	1	0.38	0.5367		
Generation	4	3.04	0.7469		
Individual	135	0.94	0.6294		
Side	1	0.48	0.4886		
Individual*side	135	1.00	0.5029		
Error	272				
Shape					
Tempreratura	26	33.83	<0.0001	0.90	<0.0001
Generation	104	1.95	<0.0001	1.34	<0.0001
Individual	3406	3.15	<0.0001	16.58	<0.0001
Side	26	0.92	0.5838	0.23	0.2174
Individual*side	3510	2.70	<0.0001	14.04	<0.0001
Error	7072				

*Pillai's tr: Pillai's Trace is a statistic that is commonly used to assess the overall significance of the differences among group means in multivariate analysis of variance (MANOVA)

Figures captions

Figure 1. Linear and morphometric characters of *Drosophila suzukii*. **A.** Measurement of the dorsal thorax. **B.** Measurement of the left lateral thorax. **C.** Wing measurements: width, length and total area. **D.** Measurement of the male spot. **E.** Measurement of the ovipositor valve and number of teeth. **F.** Landmarks used for MG measurements.

Figure 2. Half-life (TL50) of *Drosophila suzukii* adults exposed to four constant temperatures. **A.** Females; **B.** Males. Holm-Sidak method test of means, different letters with significant differences (Log-rank test $P<0.05$).

Figure 3. Effect of the four temperatures (T_{15}; T_{20}; T_{25}; T_{30}) on the development of *Drosophila suzukii*. **A.** Averages (μ ± SE) of the mean egg-to-adult duration time. Different letters present significant statistical differences in Dunn's Method ($P<0.05$). **B.** Averages (μ ± SE) of the mean estimated body weights of freshly obtained adult females and males of D. suzukii, exposed to T_{20} (green color) and T_{25} (red color). Holm-Sidak method average comparison test ($P<0.05$). *Statistically significant differences ($P<0.001$).

Figure 4. Mean (μ ± SEM) of thorax, wing, and ovipositor measurements of *Drosophila suzukii* adults reared at constant temperatures (T_{20}; T_{25}). Measurements were performed on the left wing. The color represents the temperature T_{20} green, T_{25} red, and the symbol is the sex. Circle: females; square: males. **A**. wing width (mm). **B**. Wing length (mm). **C**. Total area (mm^2). **D**. Dorsal thorax (mm^2). **E**. Left lateral thorax (mm). **F**. Left valve of the ovipositor (mm). **G**. Number of teeth of the valve. **H.** area spot of the left and right wing of the male (mm^2). Means with asterisks show statistically significant differences, Tukey/Holm-Sidak method test ($P<0.05$).

Figure 5. Effect of temperature on morphometrics of *Drosophila suzukii*. Principal component analysis of wing shape, **A**. Females; **B**. Males. Analysis of canonical wing shape variables, **C**. Females; **D**. Males. Wing shape variations, **E**. Females; **F**. Males.

Figure 1

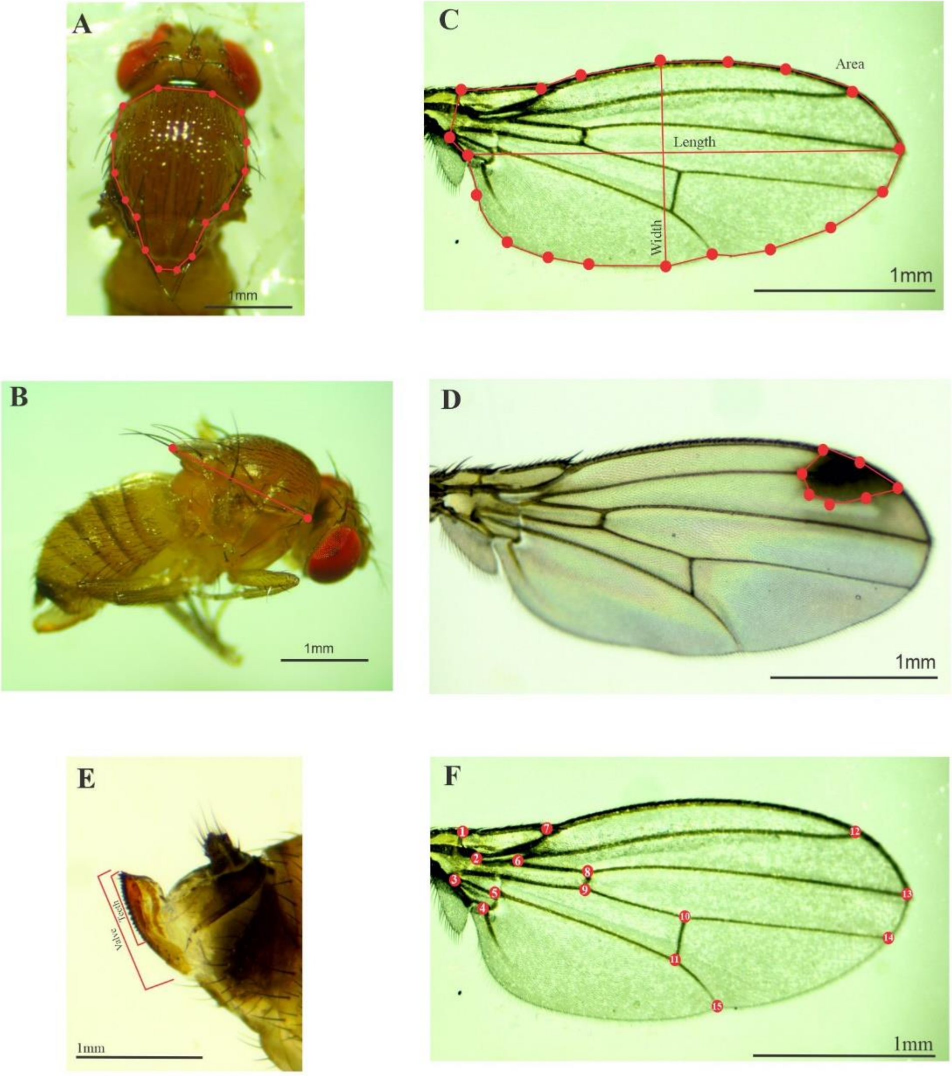

A
1mm
C
Area
Length
Width
1mm
B
1mm
D
1mm
E
Tooth
Valve
1mm
F
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
1mm

Figure 2

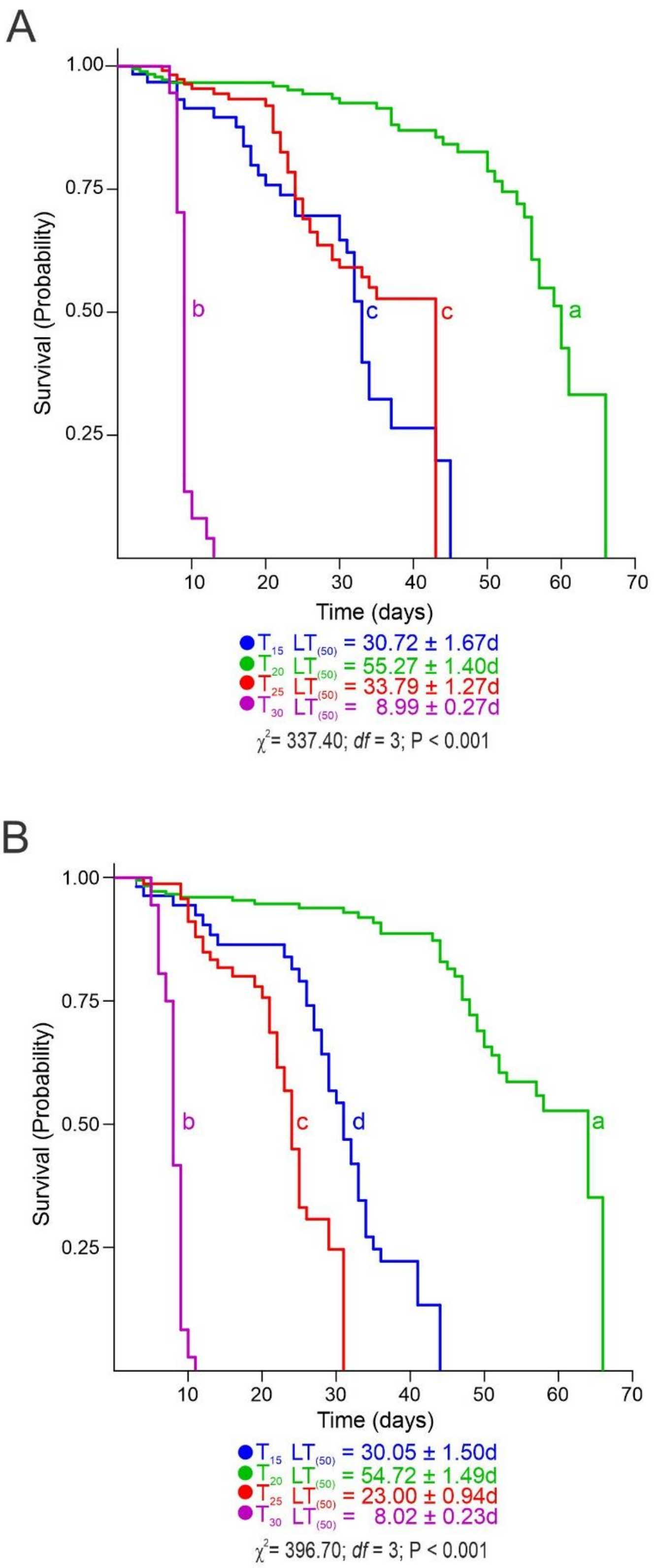

A
1.00
0.75
0.50
0.25
Survival (Probability)
b
c
c
a
10
20
30
40
50
60
70
Time (days)
● T_{15} $LT_{(50)}$ = 30.72 ± 1.67d
● T_{20} $LT_{(50)}$ = 55.27 ± 1.40d
● T_{25} $LT_{(50)}$ = 33.79 ± 1.27d
● T_{30} $LT_{(50)}$ = 8.99 ± 0.27d
χ^2= 337.40; df = 3; P < 0.001
B
1.00
0.75
0.50
0.25
Survival (Probability)
b
c
d
a
10
20
30
40
50
60
70
Time (days)
● T_{15} $LT_{(50)}$ = 30.05 ± 1.50d
● T_{20} $LT_{(50)}$ = 54.72 ± 1.49d
● T_{25} $LT_{(50)}$ = 23.00 ± 0.94d
● T_{30} $LT_{(50)}$ = 8.02 ± 0.23d
χ^2= 396.70; df = 3; P < 0.001

Figure 3

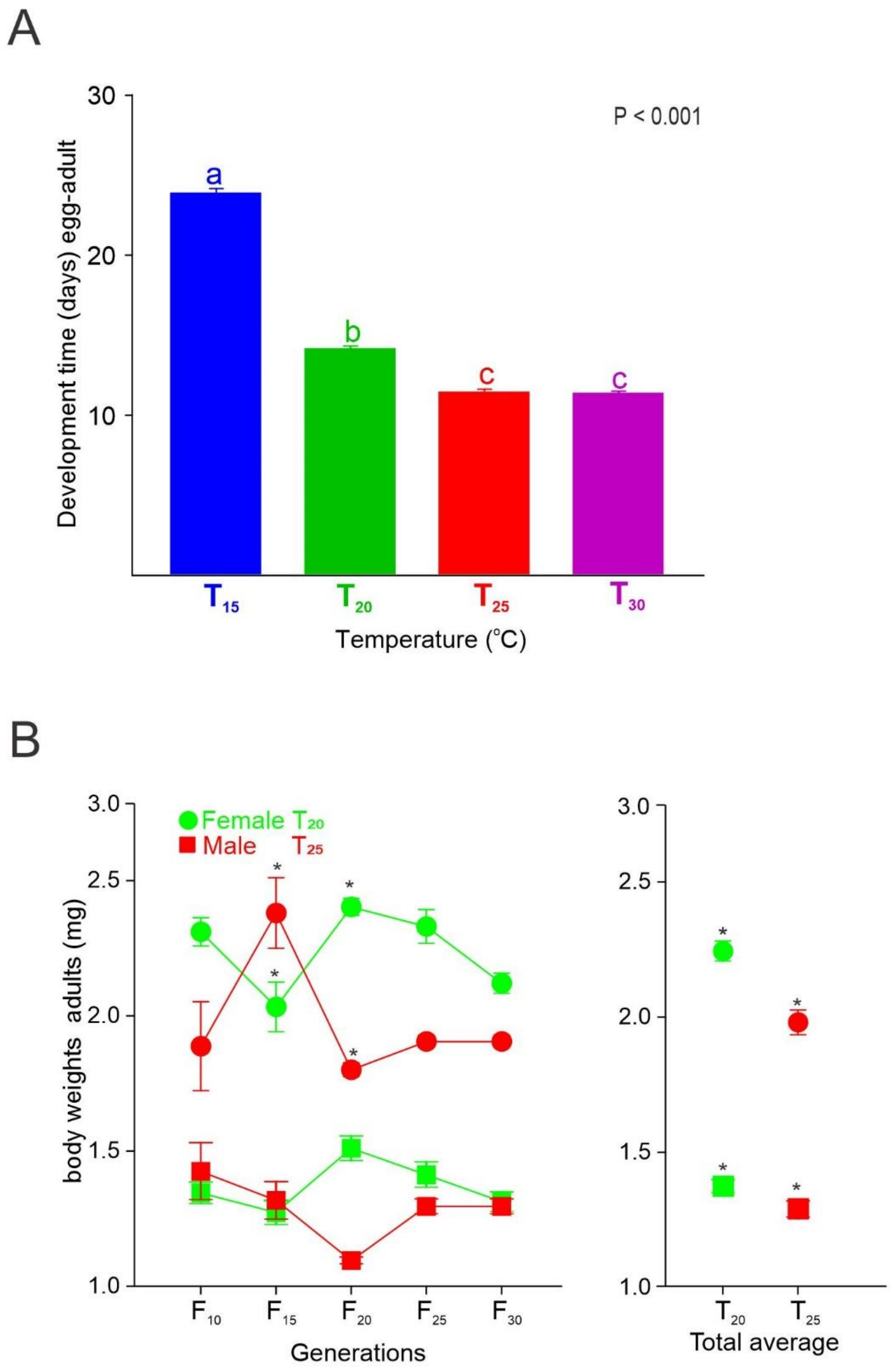

A
30
20
10
Development time (days) egg-adult
P < 0.001
a
b
c
c
T15
T20
T25
T30
Temperature (°C)
B
3.0
2.5
2.0
1.5
1.0
body weights adults (mg)
● Female T20
■ Male T25
*
F10
F15
F20
F25
F30
Generations
T20
T25
Total average

Figure 4

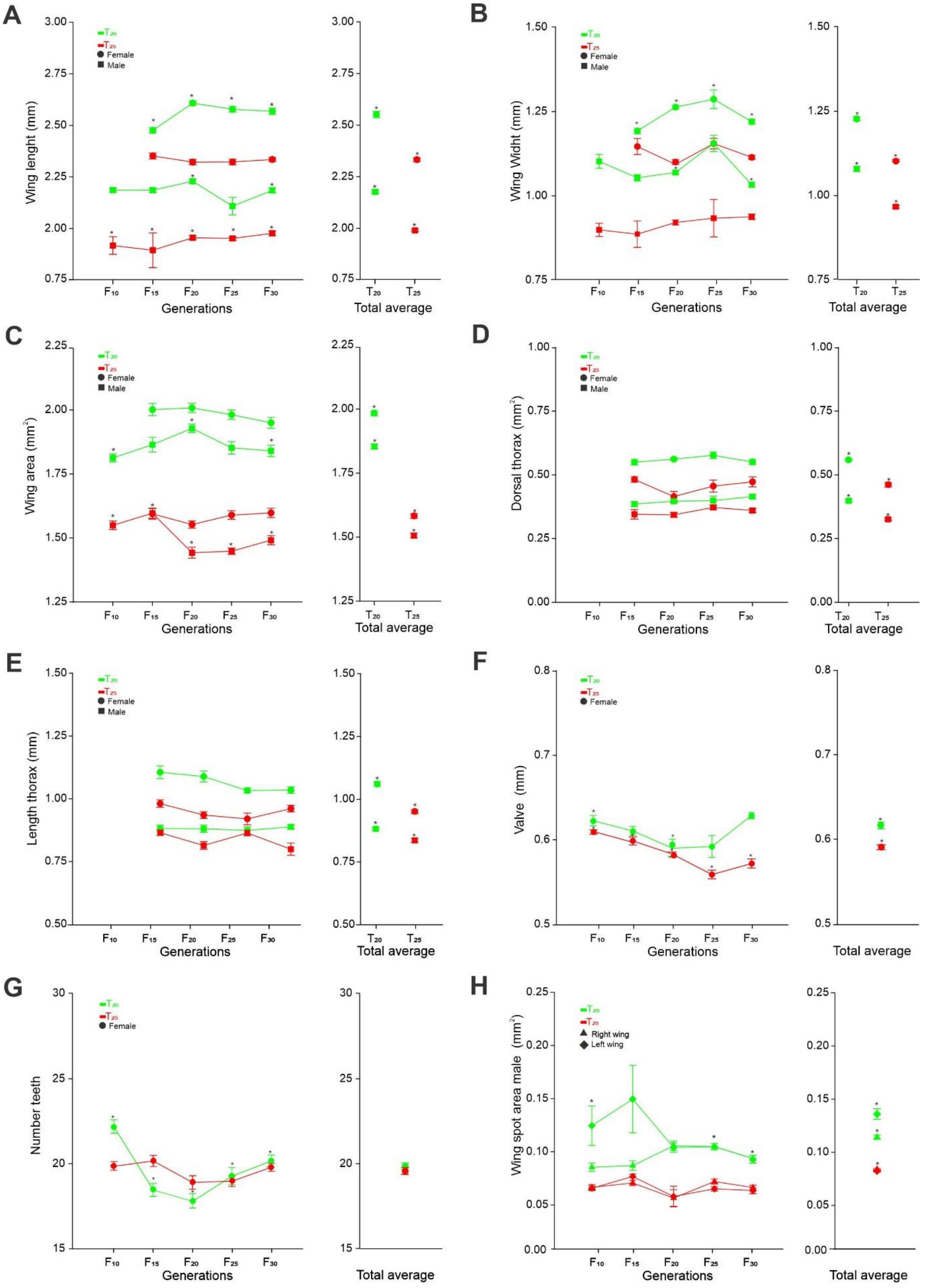
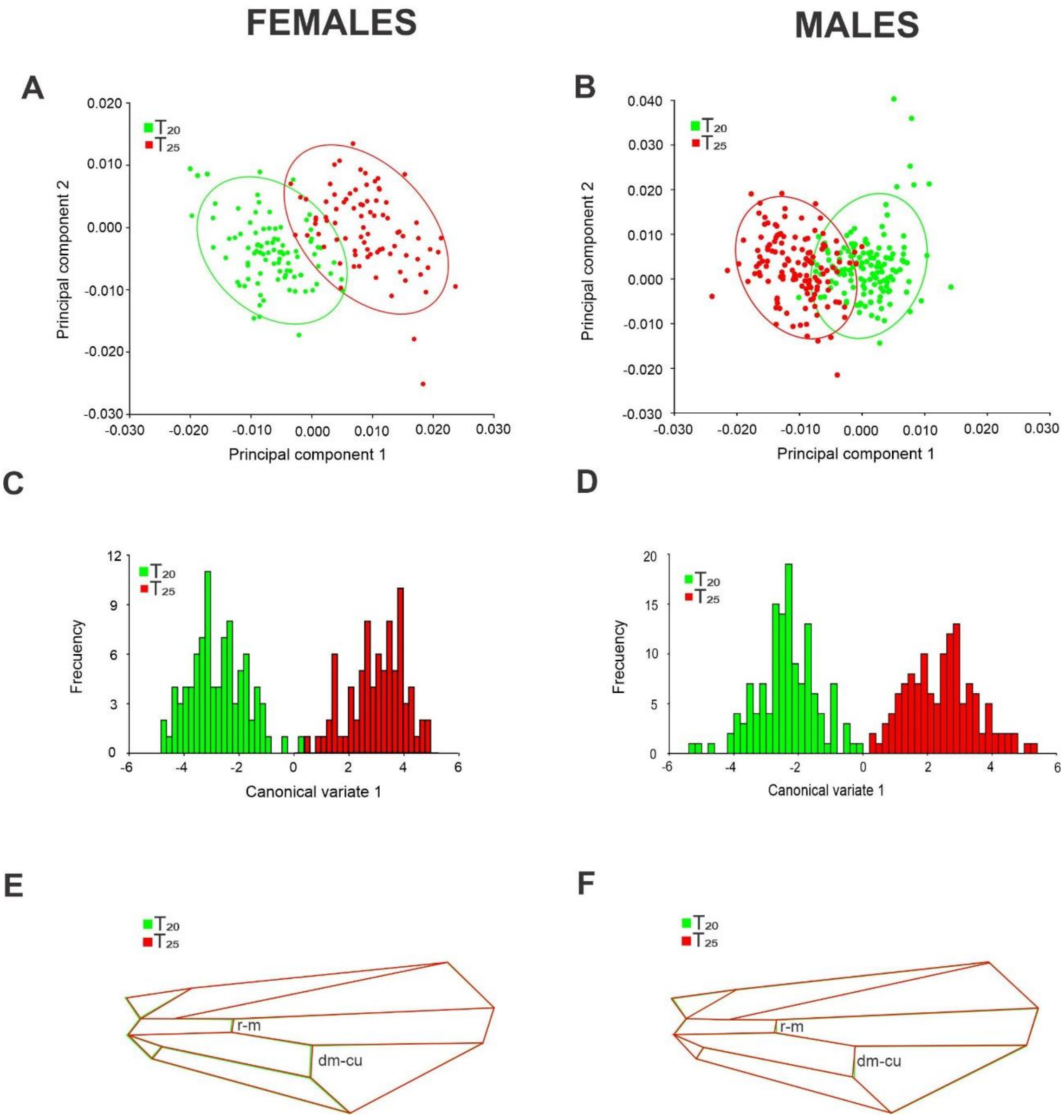
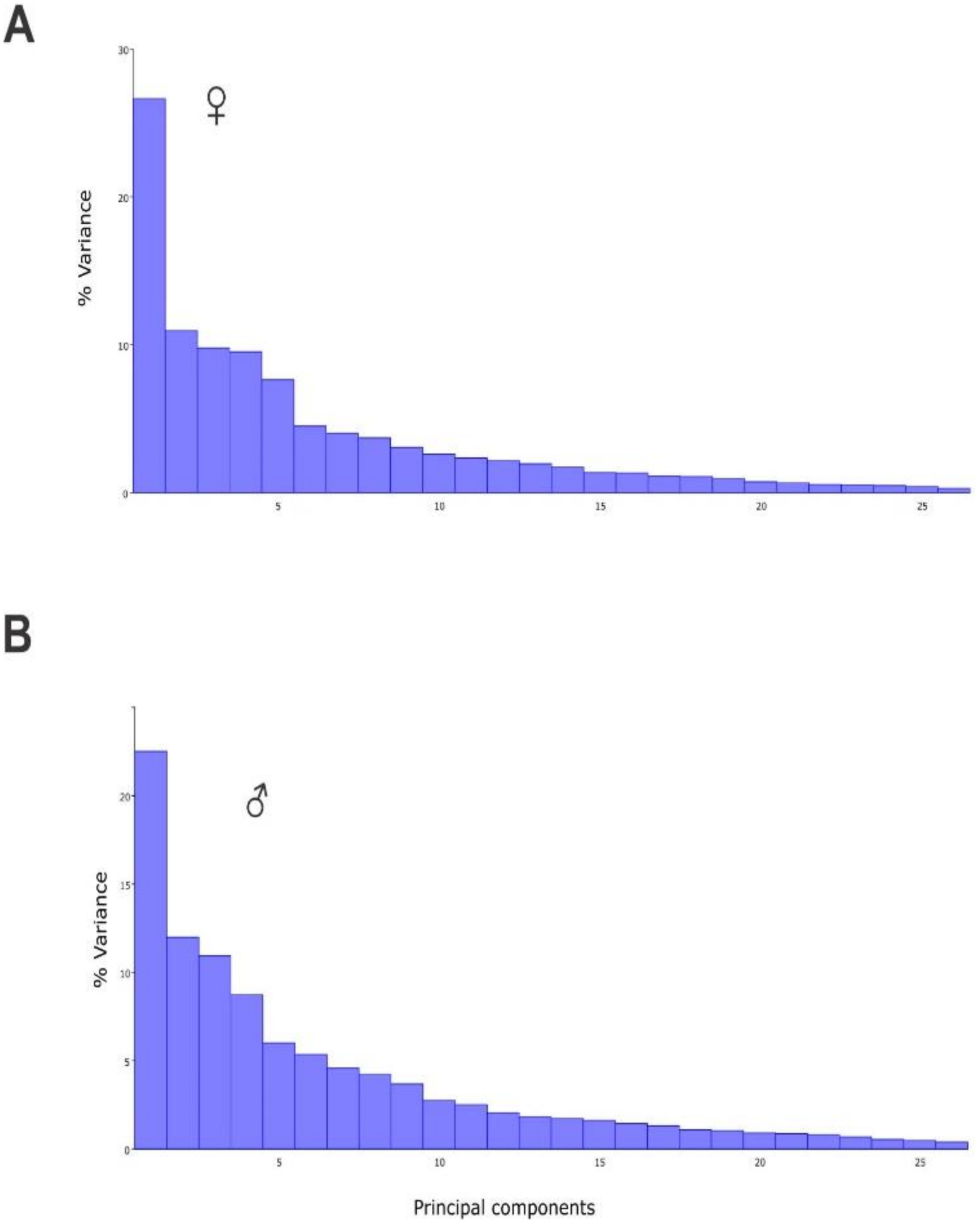

A
Wing lenght (mm)
3.00
2.75
2.50
2.25
2.00
0.75
F10 F15 F20 F25 F30
Generations
T20 T25
Total average
T20
T25
Female
Male
B
Wing Widht (mm)
1.50
1.25
1.00
0.75
C
Wing area (mm²)
2.25
2.00
1.75
1.50
1.25
D
Dorsal thorax (mm²)
1.00
0.75
0.50
0.25
0.00
E
Length thorax (mm)
1.50
1.25
1.00
0.75
0.50
F
Valve (mm)
0.8
0.7
0.6
0.5
G
Number teeth
30
25
20
15
H
Wing spot area male (mm²)
0.25
0.20
0.15
0.10
0.05
0.00
Right wing
Left wing

Figure 5


FEMALES
MALES
A
B
T20
T25
Principal component 2
Principal component 1
0.020
0.010
0.000
-0.010
-0.020
-0.030
-0.030
-0.020
-0.010
0.000
0.010
0.020
0.030
0.040
0.030
0.020
0.010
0.000
-0.010
-0.020
-0.030
C
D
Frecuency
Canonical variate 1
12
9
6
3
0
20
15
10
5
-6
-4
-2
0
2
4
6
E
F
r-m
dm-cu

Supplementary figure 1



Supplementary material

Table 1. Effect of temperature at the transgenerational level on adult body mass of *Drosophila Suzukii.*

Source of variation	**DF**	**SS**	**MS**	**F**	**P**
Female					
Temperature	1	0.483	0.483	6.606	0.015*
Generation	4	0.199	0.0499	0.682	0.610^{ns}
Residual	34	2.488	0.0732		
Total	39	3.381	0.0867		
Male					
Temperature	1	0.451	0.451	1.625	0.211^{ns}
Generation	4	0.0502	0.0125	0.452	0.770^{ns}
Residual	34	0.943	0.0277		
Total	39	1.066	0.0273		

* There is a statistically significant difference (P <0.015). *ns* Not significant

Analysis of variance Two Way ANOVA for the effect of temperature and sex level on the adult body mass of *Drosophila Suzukii.*

Source of variation	**DF**	**SS**	**MS**	**F**	**P**
Temperature	1	0.607	0.607	12.540	<0.001*
Sex	1	12.145	12.145	250.781	<0.001*
Residual	76	3.680	0.0484		
Total	79	16.592	0.210		

* There is a statistically significant difference (P <0.005)

Table 2. Evaluation of the Influence of Temperature and Sex in the width of the left wing of *Drosophila suzukki.*

Source of variation	DF	SS	MS	F	P
Temperature	1	0.983	0.983	156.36	<0.001*
Sexo	1	1.621	1.621	257.98	<0.001*
Residual	234	1.470	0.0062		
Total	237	4.158	0.0175		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and Sex in the length of the left wing of *Drosophila suzukki.*

Source of variation	DF	SS	MS	F	P
Temperature	1	2.428	2.428	261.90	<0.001*
Sexo	1	7.473	7.473	806.07	<0.001*
Residual	235	2.179	0.00927		
Total	238	12.134	0.0510		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and Sex in the area of the left wing of *Drosophila suzukki.*

Source of variation	DF	SS	MS	F	P
Temperature	1	8.029	8.029	857.59	<0.001*
Sexo	1	0.607	0.607	64.80	<0.001*
Residual	234	2.191	0.0093		
Total	236	10.946	0.0462		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and Sex on thorax dorsal of *Drosophila suzukki.*

Source of variation	DF	SS	MS	F	P
Temperature	1	0.206	0.206	40.715	<0.001*
Sexo	1	0.829	0.829	163.735	<0.001*
Residual	197	0.998	0.0050		
Total	199	2.093	0.0105		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and Sex in the length of the left of the thorax of *Drosophila suzukii.*

Source of variation	DF	SS	MS	F	P
Temperature	1	0.288	0.288	39.460	<0.001*
Sexo	1	1.150	1.150	157.90	<0.001*
Residual	197	1.435	0.0072		
Total	199	2.917	0.0146		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and and generation of effect on Thorax dorsal e length of the left in females of *D. suzukii.*

Source of variation	DF	SS	MS	F	P
Thorax dorsal					
Temperature	1	0.257	0.257	35.70	<0.001*
Generation	4	0.217	0.054	7.524	<0.001*
Residual	91	0.655	0.007		
Total	96	1.181	0.012		
Thorax left					
Temperature	1	0.325	0.325	34.81	<0.001*
Generation	4	0.940	0.235	25.16	<0.001*
Residual	91	0.850	0.009		
Total	96	2.236	0.023		

* There is a statistically significant difference (P <0.005)

Evaluation of the Influence of Temperature and and generation of effect on Thorax dorsal e length of the left in males of *D. suzukii.*

Source of variation	DF	SS	MS	F	P
Thorax dorsal					
Temperature	1	0.0343	0.0343	11.016	0.001*
Generation	3	0.0042	0.0014	0.458	0.0712 ^{ns}
Residual	97	0.302	0.0031		
Total	104	0.349	0.0033		
Thorax left					
Temperature	1	0.0514	0.0514	10.716	0.001*
Generation	3	0.0173	0.0057	1.202	0.313 ^{ns}
Residual	97	0.465	0.0048		
Total	104	0.566	0.0054		

* There is a statistically significant difference (P <0.005)

Table 3. Evaluation of the Influence of Temperature at a transgenerational level on the length of the left valve and number of teeth in *Drosophila suzukii* females.

Source of variation	DF	SS	MS	F	P
Valva					
Temperature	1	0.0232	0.0232	15.455	<0.001*
Generation	4	0.0379	0.0094	6.318	<0.001*
Residual	126	0.189	0.0015		
Total	131	0.261	0.0019		
Teeth					
Temperature	1	0.0572	0.0572	0.0155	0.901^{ns}
Generation	4	159.73	39.933	10.841	<0.001*
Residual	126	464.13	3.684		
Total	131	711.34	5.269		

* There is a statistically significant difference (P <0.005)

Table 4. Evaluation of the Influence of Temperature on the spot area on wings of male *Drosophila suzukii*.

Source of variation	DF	SS	MS	F	P
Spot Wing Male					
Temperature	1	0.1370	0.1370	23.401	<0.001*
Side	1	0.0113	0.0113	1.923	0.167
Temp*Side	1	0.0127	0.0127	2.164	0.142
Residual	283	1.662	0.0058		
Total	286	1.824	0.0063		

* There is a statistically significant difference (P <0.005)

TABLE 5. Evaluation of the effect of Temperature on the morphology of adult *Drosophila suzukii* females exposed to two temperatures. Means and standard deviations (μ ± SE) of Performance Scores. Only the left wing was considered for statistical analysis. Taking into account that the analysis of fluctuating asymmetry showed that there was no statistical difference between the wings of the same individual. Only the measurement of the ovipositor is shown. only those of the left valve are shown, previous analyses showed no statistical differences in the measurements of the two valves. The number of teeth was counted only in the sclerotized part of the valve.

		Generation[b]					
T[a]	**Variable**	**F_{10}**	**F_{15}**	**F_{20}**	**F_{25}**	**F_{30}**	**Total average**[c]
T_{20}	Length wing (mm)	-	2.476 ± 0.046*	2.608 ± 0.0395*	2.578 ± 0.047*	2.568 ± 0.0539*	2.577 ± 0.0616*
	Width wing (mm)	-	1.187 ± 0.0271*	1.270 ± 0.0250*	1.234 ± 0.041*	1.222 ± 0.0340*	1.229 ± 0.0415*
	Total wing área (mm^2)	-	2.000 ± 0.0810	2.007 ± 0.0627	1.981 ± 0.063	1.949 ± 0.0750	1.985 ± 0.0255*
	Thorax (mm^2)	-	0.549 ± 0.0375	0.561 ±0.0179	0.576 ± 0.0443	0.550 ± 0.0350	0.559 ± 0.0328*
	Thorax left (mm)	-	1.105 ± 0.087	1.088 ± 0.07425	1.033 ± 0.0347	1.0349 ± 0.0447	1.034 ±0.0592*
	Valva left (mm)	0.6303 ± 0.0292*	0.617 ± 0.0193	0.594 ± 0.0409*	0.596 ± 0.0496	0.6371 ± 0.0146	0.616 ± 0.0283*
	Teeth (number)	22.444 ± 1.888*	18.250 ± 1.4583*	17.50 ± 2.000*	19.167 ± 1.9720*	20.167 ± 1.3611*	19.10 ± 2.2017
T_{25}	Length wing (mm)	-	2.352 ± 0.051	2.322 ± 0.0452	2.323 ± 0.0462	2.334 ± 0.0343	2.331 ± 0.0444*
	Width wing (mm)	-	1.105 ± 0.0285	1.087 ± 0.0256	1.120 ± 0.0154	1.1090 ± 0.022	1.105 ± 0.0255*
	Total wing área (mm^2)	-	1.593 ± 0.071	1.552 ± 0.049	1.589 ± 0.057	1.597 ± 0.0623	1.583 ± 0.0623*
	Thorax (mm^2)	-	0.482 ± 0.0382	0.414 ± 0.069	0.455 ± 0.082	0.472 ± 0.0689	0.456 ± 0.0667*
	Thorax left (mm)	-	0.981166667	0.935166667	0.919 ± 0.0809	0.961 ± 0.0449	0.949 ± 0.0615*
	Valva left (mm)	0.618 ± 0.0143*	0.603 ± 0.0143*	0.586 ± 0.0097*	0.559 ± 0.0195*	0.574 ± 0.0203*	0.581 ± 0.0290*
	Teeth (number)	19.818 ± 1.2314	20.167 ± 1.2223	18.750 ± 1.458	18.833 ± 1.194	19.750 ± 0.791	19.549 ± 1.201

[a] Constant temperature to which *D. suzukii* adults were grown. [b] Average of each variable in each generation. [c] Total average (sum of all individuals of all generations).

*There is a statistically significant difference (P <0.005)

Evaluation of the effect of Temperature on the morphology of adult *Drosophila suzukii* males exposed to two temperatures. Means and standard deviations (μ ± SE) of Performance Scores. Only the left wing was considered for statistical analysis. Taking into account that the analysis of fluctuating asymmetry showed that there was no statistical difference between the wings of the same individual.

Generation[b]							
T[a]	**Variable**	**F_{10}**	**F_{15}**	**F_{20}**	**F_{25}**	**F_{30}**	**Total average**[c]
T_{20}	Length wing (mm)	2.186 ± 0.041	2.185 ± 0.043	2.229 ± 0.037*	2.108 ± 0.158	2.185 ± 0.044*	2.178 ± 0.062*
	Width wing (mm)	1.097 ± 0.009	1.046 ± 0.035	1.062 ± 0.017*	1.151 ± 0.212	1.026 ± 0.027*	1.080 ± 0.0740*
	Total wing área (mm^2)	1.812 ± 0.043*	1.864 ± 0.068	1.928 ± 0.106*	1.851 ± 0.057	1.84 ± 0.077*	1.854 ± 0.0856*
	Spot wing right (mm^2)	0.111 ± 0.018	0.112 ± 0.016	0.133 ± 0.016	0.132 ± 0.011	0.119 ± 0.0128	0.144 ± 0.0490*
	Spot wing left (mm^2)	0.153 ± 0.088*	0.151 ± 0.119	0.131 ± 0.016	0.132 ± 0.012*	0.119 ± 0.012*	0.121 ± 0.0168*
	Thorax (mm^2)	-	0.385 ± 0.033	0.396 ± 0.020	0.398 ± 0.065	0.414 ± 0.029	0.398 ± 0.038*
	Thorax left (mm)	-	0.881 ± 0.054	0.880 ± 0.050	0.0874 ± 0.050	0.888 ± 0.030	0.880 ± 0.045*
T_{25}	Length wing (mm)	1.917 ± 0.197*	1.895 ± 0.292*	1.955 ± 0.037*	1.952 ± 0.030*	1.977 ± 0.042*	1.991 ± 0.053*
	Width wing (mm)	0.889 ± 0.089	0.857 ± 0.138	0.911 ± 0.027	0.924 ± 0.018	0.928 ± 0.030	0.916 ± 0.040*
	Total wing área (mm^2)	1.549 ± 0.0730*	1.596 ± 0.067*	1.442 ± 0.076*	1.447 ± 0.0468	1.491 ± 0.0065	1.507 ± 0.0830*
	Spot wing right (mm^2)	0.0905 ± 0.0123	0.095 ± 0.009	0.0795 ± 0.022	0.0964 ± 0.009	0.0900 ± 0.0020	0.090 ± 0.0120*
	Spot wing left (mm^2)	0.0891 ± 0.0105	0.101 ± 0.008	0.0811 ± 0.023	0.089 ± 0.008	0.087 ± 0.0120	0.0913 ± 0.0124*
	Thorax (mm^2)	-	0.345 ± 0.0642	0.3430 ± 0.033	0.372 ± 0.032	0.359 ± 0.0279	0.362 ± 0.031*
	Thorax left (mm)	-	0.864 ± 0.0037	0.8140 ± 0.0560	0.863 ± 0.0420	0.798 ± 0.0909	0.835 ± 0.063*

[a] Constant temperature to which *D. suzukii* adults were grown. [b] Average of each variable in each generation. [c] Total average (all individuals of all generations).

*There is a statistically significant difference (P <0.005).

Table 6. Procrustes ANOVA of the temperature effect on *Drosophila suzukii adults*. Sums of squares (SS) and Mean squares (MS) are units of procusters distances (dimensionless). The MS of de error are Small that individual x side, discarding measurement error of digitization of landmarks.

Effect	SS	MS	df	F	P	Pillai tr*	P (param.)
Females							
Individual	0.08713336	0.0000389684	2236	3.63	<.0001	16.96	<.0001
Side	0.00031021	0.0000119310	26	1.11	0.3173	0.31	0.4415
Ind*Side	0.02400516	0.0000107358	2236	2.54	<.0001	13.27	<.0001
Error 1	0.01910177	0.0000042223	4524				
Males							
Individual	0.15665090	0.0000446299	3510	3.15	<.0001	16.58	<.0001
Side	0.00033796	0.0000129985	26	0.92	0.5838	0.23	0.2174
Ind*Side	0.04971161	0.0000141629	3510	2.70	<.0001	14.04	<.0001
Error 1	0.03686994	0.0000052521	7072				