

FELIPE ANDREAZZA

**OCCURRENCE REPORTS AND INSECTICIDE
SUSCEPTIBILITY IN *Drosophila suzukii* POPULATIONS
FROM BRAZILIAN SOUTHEAST REGION**

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Entomology Graduate Program to obtain the title of *Magister Scientie*.

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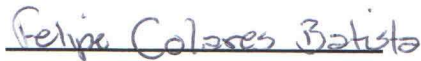
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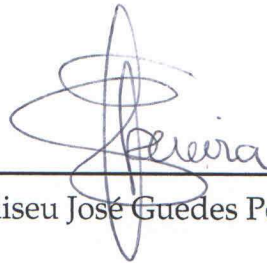
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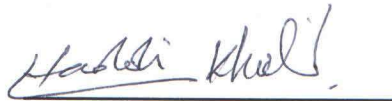
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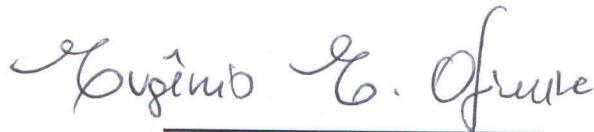
Felipe Colares Batista



Eliseu José Guedes Pereira



Haddi Khalid
(Co-Adviser)



Eugênio Eduardo de Oliveira
(Adviser)

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Abstract

ANDREAZZA, Felipe, M.Sc., Universidade Federal de Viçosa, July, 2017. **Occurrence reports and insecticide susceptibility in *Drosophila suzukii* populations from Brazilian Southeast Region.** Advisor: Eugênio Eduardo de Oliveira. Co-Advisor: Haddi khalid.

Early monitoring and toxicological studies with invasive pest species, such as the Spotted Wing *Drosophila*, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), are of importance. It took only about 3 years to *D. suzukii* become the worldwide most important pest of small soft-skinned fruits, such as strawberries, blueberries and cherries. This drosophilid lays its eggs inside undamaged fruits, and its larvae destroy the fruit tissues, making it unmarketable. While attention was driven to the search about *D. suzukii* bioecology and alternative control tools, the spray with broad-spectrum insecticides has been the most used emergency control strategy, increasing the probability of insecticide resistance selection in this species. Thus, in the current study, field surveys were performed aiming to identify and report the arrival/presence of the *D. suzukii* in two Brazilian states. Then, preliminary insecticide susceptibility comparisons were carried out on populations originally collected in these regions to evidence potential resistance sources. Finally, it was assessed the toxicity of lime-sulfur (i.e. an old alternative pesticide) on *D. suzukii* and its oviposition and development on sprayed strawberry plants. The field surveys demonstrated that *D. suzukii* is already present in both Minas Gerais (MG) and Espírito Santo (ES) states. The species was first collected in a strawberry field at the municipality of Ervália - MG in March of 2016 and in Juiz de Fora - MG in November of 2016. Later, in February of 2017, the species was also found infesting strawberry and blackberry fields at the municipalities of Domingos Martins and Santa Maria de Jetibá - ES. In the blackberry fields, the infestation of *D. suzukii* occurred simultaneously with its confamilial, *Zaprionus indianus* Gupta (Diptera: Drosophilidae). The susceptibility comparisons among *D. suzukii* populations identified a potential source of resistance to imidacloprid in a population originally collected at the municipality of Juiz de Fora - MG ($P < 0.05$), while no consistent differences among populations were found for permethrin ($P > 0.05$). Lime-sulfur was toxic ($LC_{50} = 26.6$ mL/L) to *D. suzukii* flies in low concentrations, and its spray on strawberry plants reduced the *D. suzukii* oviposition in both choice (48.6% of reduction) and no choice (47.6% of reduction) semi-field bioassays ($P < 0.05$). It was also found a slight delay in the pre-imaginal developmental time of individuals from eggs laid on lime-sulfur treated plants only in the choice bioassay, indicating a possible effect of the females oviposition choice on its offspring development. Two other alternative preparations based in mixture of sulfur, lime, potassium permanganate, salt and detergent and the insecticides azadirachtin and indoxacarb did not cause high *D. suzukii* mortalities. As conclusions, *D. suzukii* has extended its current distribution range in Brazil, with presence of resistance sources to imidacloprid. Alter-

natives, such as lime-sulfur, might be recommended in rotations for both traditional and organic farming approaches. Further studies monitoring the occurrence of insecticide resistance in field populations, as well as better investigation of the sublethal effects and compatibility of lime-sulfur applications on other *D. suzukii* hosts should be performed.

Resumo

ANDREAZZA, Felipe, M.Sc., Universidade Federal de Viçosa, julho de 2017. **Relatos de ocorrência e susceptibilidade à inseticidas em populações de *Drosophila suzukii* da região sudeste Brasileira.** Orientador: Eugênio Eduardo de Oliveira. Coorientador: Haddi khalid.

O monitoramento precoce e estudos toxicológicos com espécies-praga invasoras, como a Drosófila das Asas Manchadas, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), são de importância. Levou apenas cerca de 3 anos para *D. suzukii* se tornar mundialmente a mais importante praga de pequenas frutas, como os morangos, mirtilos e cerejas. Este drosofilídeo coloca seus ovos dentro de frutas sadias, e suas larvas destroem os tecidos destas frutas, tornando-as não comercializáveis. Enquanto a atenção tem se dirigido à pesquisas sobre a bioecologia de *D. suzukii* e ferramentas de controle alternativas, a aplicação com inseticidas de amplo espectro tem sido a estratégia de controle emergencial mais utilizada, aumentando a probabilidade de seleção de resistência aos inseticidas nesta espécie. Assim, no presente estudo foram realizadas coletas de campo visando identificar e relatar a chegada/presença de *D. suzukii* em dois estados brasileiros. Em seguida, realizou-se comparações preliminares de susceptibilidade à inseticidas em populações originalmente coletadas nessas regiões para evidenciar potenciais fontes de resistência. Finalmente, foi avaliada a toxicidade da calda sulfocálcica (i.e. um antigo pesticida alternativo) em *D. suzukii* e na sua oviposição e desenvolvimento em plantas de morango tratadas. As coletas de campo demonstraram que *D. suzukii* já está presente nos estados de Minas Gerais (MG) e Espírito Santo (ES). A espécie foi coletada pela primeira vez em uma produção de morangos no município de Ervália - MG em março de 2016 e em Juiz de Fora - MG em novembro de 2016. Mais tarde, em fevereiro de 2017, a espécie também foi encontrada infestando produções de morango e amora nos municípios de Domingos Martins e Santa Maria de Jetibá - ES. Nos campos de amora, a infestação de *D. suzukii* ocorrera simultaneamente com o seu confamiliar *Zaprionus indianus* Gupta (Diptera: Drosophilidae). As comparações de susceptibilidade entre as populações de *D. suzukii* identificaram uma fonte potencial de resistência ao imidacloprido em uma população originalmente coletada no município de Juiz de Fora - MG ($P < 0,05$), enquanto que não foram encontradas diferenças consistentes entre as populações para a permetrina ($P > 0,05$). A calda sulfocálcica foi tóxica ($CL_{50} = 26,6$ mL/L) para *D. suzukii* em baixas concentrações, e a sua pulverização em plantas de morango reduziu a oviposição em bioensaios de semi-campo com chance de escolha (48,6% de redução) e sem chance de escolha (47,6% de redução) ($P < 0,05$). Verificou-se também um ligeiro atraso no tempo de desenvolvimento pré-imaginal dos indivíduos oriundos a partir de ovos colocados em plantas tratadas com a calda sulfocálcica apenas no bioensaio com escolha, indicando um possível efeito da escolha de oviposição das fêmeas no desenvolvimento de sua prole. Duas outras preparações alternativas à base de mistura de enxofre, cal,

permanganato de potássio, sal e detergente e os inseticidas azadiractina e indoxacarb não causaram mortalidades elevadas de *D. suzukii*. Como conclusões, *D. suzukii* ampliou sua atual faixa de distribuição no Brasil, com presença de fontes de resistência ao imidacloprido. Alternativas, como a calda sulfocálcica, pode ser recomendada em rotações para ambas as abordagens de agricultura orgânica e tradicional. Estudos adicionais que monitorem a ocorrência de resistência à inseticidas em populações de campo, bem como uma melhor investigação dos efeitos subletais e compatibilidade de aplicações de calda sulfocálcica em outros hospedeiros de *D. suzukii* devem ser realizados.

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General introduction

Invasive insect pests are a continuous threat not only to the agricultural fields but also to the native biological system balances (Pimentel et al. 2005; Campos et al. 2014; Asplen et al. 2015; Gilioli et al. 2017). Therefore, the monitoring of an invasive pest dispersion can help with the set up of the necessary strategies to manage it, from laws and regulations to field and post-harvest control or trade market barriers (Desneux et al. 2011; Cini et al. 2012; Follett et al. 2014; Kriticos et al. 2015). The monetary losses an exotic pest invasion can cause, often together with common lack of knowledge about its bioecology and possible management strategies, make the chemical control the prevalent approach, because of its fast response. This ends up with selection of insecticide resistant populations because of the indiscriminate use of insecticides as emergency control strategy (Campos et al. 2014; Mishra et al. 2017; Voudouris et al. 2017).

The selection of resistant populations is a common phenomenon among invasive pest species (Haddi et al. 2012; Campos et al. 2014; Roditakis et al. 2015; Haddi et al. 2017; Kaplanoglu et al. 2017). In some cases the pest species population have already been selected for traits conferring resistance to some insecticides even before their field application at commercial levels (Sial et al. 2010). The velocity a resistance can be selected depends on several factors. The ones associated to the pests, as short generation time and high reproductive rate are usually common among the invasive species (Campos et al. 2014; Emiljanowicz et al. 2014). On the other hand, some management strategies can contribute or delay its occurrence. Since the intensive insecticide application is the most common approach used against those pests, a panorama for a fast insecticide selection is usually created (Mishra et al. 2017).

The cases of insecticide resistance reported in the literature cover several mechanism from target site mutation (Haddi et al. 2017) to metabolic detoxification (either

throughout point mutation in the encoding gene of detoxification enzymes or its over-expression) (Bogwitz et al. 2005; Puinean et al. 2010; Kaplanoglu et al. 2017), cuticular reduced absorbance (Silva et al. 2012) and avoidance (Boonyuan et al. 2016). These mechanisms can occur alone, or associated and confer resistance either to a single insecticide mode of action (Haddi et al. 2017), or to several ones (Daborn et al. 2001; Dunley et al. 2006). Thus, the monitoring of insecticide susceptibility differences among field population of an invasive species is indispensable.

In a very rapid dispersion (i.e. less than 3 years), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive species from Asia, become the most important pest in both North America and Europe small fruit production fields (Hauser 2011; Asplen et al. 2015; Cini et al. 2014). The females of this drosophilid have evolved the capacity to oviposit inside healthy undamaged fruits (Atallah et al. 2014), especially the soft-skinned berries, as strawberries, blueberries, blackberries and cherries (Asplen et al. 2015). The damage caused by the *D. suzukii* larvae feeding on the fruit tissues impairs even higher direct economic losses if compared with other small fruit herbivorous pests (Bernardi et al. 2015). Currently, the species could be partially managed by the combined use of exclusion netting, mass trapping and pupal parasitoids (Kawase et al. 2007; Chabert et al. 2012; Hampton et al. 2014; Daane et al. 2016), but the association of its highly damaging potential with lack of knowledge on its bioecology and management still forces the growers to use proactive insecticides sprays as the main strategy to control this drosophilid pest (Van Timmeren and Isaacs 2013; Haye et al. 2016). Furthermore, the zero tolerance politics of some regions, increases the insecticide application as preventive tool, and hence, the insecticide selection pressure (Van Timmeren and Isaacs 2013; Mishra et al. 2017).

Several studies worldwide provided preliminary screening of insecticide efficacy on *D. suzukii* populations from three major invaded continents (i.e. North and South America and Europe) (Bruck et al. 2011; Haviland and Beers 2012; Van Timmeren and Isaacs 2013; Cuthbertson et al. 2014; Andreazza et al. 2017). These studies testing insecticide with different mode of action showed that broad spectrum insecticides, as organophosphates, pyrethroids, neonicotinoids and spinosyns are the most effective against this pest and gives a good source of rotations options to delay any resistance selection. Nevertheless, studies looking for both, alternative products as an option to

organic farming [as in Erland et al. (2015); Pérez-Guerrero and Molina (2016) and Jang et al. (2017)] and baseline susceptibilities/field resistance monitoring are important. The closely related *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) had been selected to insecticide resistance faster than predicted by models (Karasov et al. 2010), thus it can be expected that even with rotation of insecticides, the resistance selection is a time-line phenomenon upon selection (Mishra et al. 2017).

In Brazil, *D. suzukii* was first reported in 2013 (Deprá et al. 2014) and economic damages to strawberries were reported within only one year from this pest invasion, in southern Brazil (Santos 2014). The current distribution of this pest in the Brazilian territory includes the entire south region (Deprá et al. 2014; Geisler et al. 2015), the states of São Paulo and Rio de Janeiro in southeast region (Bitner-Mathé et al. 2014; Vilela and Mori 2014), and the state of Goiás, in the central region of Brazil (Paula et al. 2014).

Minas Gerais (MG) and Espírito Santo (ES) states remains as the only two states from the southeast Brazilian region where *D. suzukii* is still not reported, but analyzing its invasion history in other countries (Hauser 2011; Cini et al. 2012) it is possible to predict this pest invasion as unavoidable. Furthermore, studies had predicted the potential distribution of this pest within these two Brazilian states (Benito et al. 2016). Thus, since both states cultivate a large amount of important *D. suzukii* main hosts, especially strawberries (Incapere 2010; Silveira and Guimarães 2014), in the current study, we firstly aimed to identify and report the presence of this pest in small berries fields in both, the Zona da Mata Mineira (MG) and Capixaba Highlands regions (ES), what was accomplished in the studies described in chapters 1 and 2. Then, as described in chapter 3, insecticide susceptibility comparisons were performed on two *D. suzukii* populations originally collected in MG state and on a population maintained in laboratory culture for 48 generations (i.e. a insecticide susceptibility pattern population), evidencing potential sources of insecticide resistance in this species. Finally, in chapter 4, a set of preliminary toxicological studies was carried out on the insecticide susceptibility pattern population, testing alternative products that could be used to manage this pest in either conventional or organic farming.

References

- Andreazza F, Bernardi D, Baronio CA, Pasinato J, Nava DE, Botton M (2017) **Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae).** *Pest Management Science* 73:146–152.
- Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Kárpáti Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Vétek G, Vogt H, Walton VM, Yu Y, Zappalà L, Desneux N (2015) **Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities.** *Journal of Pest Science* 88:469–494.
- Atallah J, Teixeira L, Salazar R, Zaragoza G, Kopp A (2014) **The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species.** *Proceedings of the Royal Society of London B: Biological Sciences* 281:2814.
- Benito NP, Lopes-da Silva M, Santos RSSd (2016) **Potential spread and economic impact of invasive *Drosophila suzukii* in Brazil.** *Pesquisa Agropecuária Brasileira* 51:571–578.
- Bernardi D, Botton M, Nava DE, Zawadneak MAC (2015) *Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro*, Vol. 1 Embrapa, Brasília - DF.
- Bitner-Mathé BC, Victorino J, Faria FS (2014) ***Drosophila suzukii* has been found in tropical Atlantic Rainforest in southeastern Brazil.** *Drosophila Information Service* 97:136–137.
- Bogwitz MR, Chung H, Magoc L, Rigby S, Wong W, O’Keefe M, McKenzie JA, Batterham P, Daborn PJ (2005) **Cyp12a4 confers lufenuron resistance in a natural population of *Drosophila melanogaster*.** *Proceedings of the National Academy of Sciences* 102:12807–12812.
- Boonyuan W, Bangs MJ, Grieco JP, Tiawsirisup S, Prabaripai A, Chareonviriyaphap T (2016) **Excito-repellent responses between *Culex quinquefasciatus* permethrin susceptible and resistant mosquitoes.** *Journal of Insect Behavior* 29:415–431.
- 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.
- Campos MR, Rodrigues ARS, Silva WM, Silva TBM, Silva VRF, Guedes RNC, Siqueira HAA (2014) **Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance.** *Plos ONE* 9:e103235.
- Chabert S, Allemand R, Poyet M, Eslin P, Gibert P (2012) **Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, *Drosophila suzukii*.** *Advances in Parasitology* 63:40–47.
- Cini A, Anfora G, Escudero-Colomar LA, Grassi A, Santosuosso U, Seljak G, Papini A (2014) **Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe.** *Journal of Pest Science* 87:559–566.

- Cini A, Ioriatti C, Anfora G (2012) **A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management.** *Bulletin of Insectology* 65:149–160.
- Cuthbertson AG, Collins DA, Blackburn LF, Audsley N, Bell HA (2014) **Preliminary screening of potential control products against *Drosophila suzukii*.** *Insects* 5:488–498.
- Daane KM, Wang XG, Biondi A, Miller B, Miller JC, Riedl H, Shearer PW, Guerrieri E, Giorgini M, Buffington M, van Achterberg K, Song Y, Kang T, Yi H, Jung C, Lee DW, Chung BK, Hoelmer KA, Walton VM (2016) **First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents.** *Journal of Pest Science* 89:823–835.
- Daborn P, Boundy S, Yen J, Pittendrigh B, French Constant R (2001) **DDT resistance in *Drosophila* correlates with Cyp6g1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid.** *Molecular Genetics and Genomics* 266:556–563.
- 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, Luna MG, Guillemaud T, Urbaneja A (2011) **The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production.** *Journal of Pest Science* 84:403–408.
- Dunley JE, Brunner JF, Doerr MD, Beers E (2006) **Resistance and cross-resistance in populations of the leafrollers, *Choristoneura rosaceana* and *Pandemis pyrusana*, in Washington apples.** *Journal of Insect Science* 6:14.
- Emiljanowicz LM, Ryan GD, Langille A, Newman J (2014) **Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet.** *Journal of Economic Entomology* 107:1392–1398.
- Erland LAE, Rheault MR, Mahmoud SS (2015) **Insecticidal and oviposition deterrent effects of essential oils and their constituents against the invasive pest *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae).** *Crop Protection* 78:20–26.
- Follett PA, Swedman A, Price DK (2014) **Postharvest irradiation treatment for quarantine control of *Drosophila suzukii* (Diptera: Drosophilidae) in fresh commodities.** *Journal of Economic Entomology* 107:964–969.
- Geisler FCS, Santos J, Holdefer DR, Garcia FRM (2015) **Primeiro registro de *Drosophila suzukii* (Matsumura, 1931)(Diptera: Drosophilidae) para o estado do Paraná, Brasil e de novos hospedeiros.** *Revista de Ciências Ambientais* 9:125–129.
- Gilioli G, Schrader G, Carlsson N, van Donk E, van Leeuwen CH, Martín PR, Pasquali S, Vilà M, Vos S (2017) **Environmental risk assessment for invasive alien species: A case study of apple snails affecting ecosystem services in Europe.** *Environmental Impact Assessment Review* 65:1–11.
- Haddi K, Berger M, Bielza P, Rapisarda C, Williamson MS, Moores G, Bass C (2017) **Mutation in the ace-1 gene of the tomato leaf miner (*Tuta absoluta*) associated with organophosphates resistance.** *Journal of Applied Entomology* p. 10.1111/jen.12386.

- Haddi K, Berger M, Bielza P, Cifuentes D, Field LM, Gorman K, Rapisarda C, Williamson MS, Bass C (2012) **Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*).** *Insect Biochemistry and Molecular Biology* 42:506–513.
- Haddi K, Tomé HVV, Du Y, Valbon WR, Nomura Y, Martins GF, Dong K, Oliveira EE (2017) **Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of *Aedes aegypti*: a potential challenge for mosquito control.** *Scientific Reports* 7:46549.
- Hampton E, Koski C, Barsoian O, Faubert H, Cowles RS, Alm SR (2014) **Use of early ripening cultivars to avoid infestation and mass trapping to manage *Drosophila suzukii* (Diptera: Drosophilidae) in *Vaccinium corymbosum* (Ericales: Ericaceae).** *Journal of Economic Entomology* 107:1849–1857.
- Hauser M (2011) **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* 67:1352–1357.
- Haviland DR, Beers EH (2012) **Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries.** *Journal of Integrated Pest Management* 3:F1–F6.
- Haye T, Girod P, Cuthbertson AGS, Wang XG, Daane KM, Hoelmer KA, Baroffio C, Zhang JP, Desneux N (2016) **Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world.** *Journal of Pest Science* 89:643–651.
- Incaper (2010) **Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER): 10 anos de integração pesquisa-extensão.** *Incaper em Revista* 1:145.
- Jang M, Kim J, Yoon KA, Lee SH, Park CG (2017) **Biological activity of Myrtaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae).** *Pest Management Science* 73:404–409.
- Kaplanoglu E, Chapman P, Scott IM, Donly C (2017) **Overexpression of a cytochrome P450 and a UDP-glycosyltransferase is associated with imidacloprid resistance in the Colorado potato beetle, *Leptinotarsa decemlineata*.** *Scientific Reports* 7:1762.
- Karasov T, Messer PW, Petrov DA (2010) **Evidence that adaptation in *Drosophila* is not limited by mutation at single sites.** *PLoS Genetics* 6:e1000924.
- Kawase S, Uchino K, Takahashi K (2007) **Control of cherry drosophila, *Drosophila suzukii*, injurious to blueberry.** *Plant Protection* 61:205–209.
- Kriticos DJ, Ota N, Hutchison WD, Beddow J, Walsh T, Tay WT, Borchert DM, Paula-Moreas SV, Czepak C, Zalucki MP (2015) **The potential distribution of invading *Helicoverpa armigera* in North America: is it just a matter of time?** *Plos ONE* 10:e0119618.
- Mishra R, Chiu JC, Hua G, Tawari NR, Adang MJ, Sial AA (2017) **High throughput sequencing reveals *Drosophila suzukii* responses to insecticides.** *Insect Science* n/a:10.1111/1744-7917.12498.
- Paula MA, Lopes PHS, Tidon R (2014) **First record of *Drosophila suzukii* in the Brazilian Savanna.** *Drosophila Information Service* 97:113–115.

- Pérez-Guerrero S, Molina J (2016) **Laboratory approach to the use of sulphur and kaolin as preventive control against *Drosophila suzukii***. *Spanish Journal of Agricultural Research* 14:10–01.
- Pimentel D, Zuniga R, Morrison D (2005) **Update on the environmental and economic costs associated with alien-invasive species in the United States**. *Ecological Economics* 52:273–288.
- Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS, Williamson MS, Bass C (2010) **Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae***. *Plos Genetics* 6.
- Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouil M, Bassi A (2015) **First report of *Tuta absoluta* resistance to diamide insecticides**. *Journal of Pest Science* 88:9–16.
- Santos RSS (2014) **Ocorrência de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morango no Brasil**. *Comunicado Técnico/ Embrapa Uva e Vinho* 159:4.
- Sial AA, Brunner JF, Doerr MD (2010) **Susceptibility of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) to two new reduced-risk insecticides**. *Journal of Economic Entomology* 103:140–146.
- Silva AX, Jander G, Samaniego H, Ramsey JS, Figueroa CC (2012) **Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) I: a transcriptomic survey**. *Plos ONE* 7:e36366.
- Silveira GRS, Guimarães B (2014) **Aspectos sociais e econômicos da cultura do morangueiro**. *Informe Agropecuário* 35:7–10.
- 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.
- Vilela CR, Mori L (2014) **The invasive spotted-wing *Drosophila* (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil)**. *Revista Brasileira de Entomologia* 58:371–375.
- Voudouris CC, Williamson MS, Skouras PJ, Kati AN, Sahinoglou AJ, Margaritopoulos JT (2017) **Evolution of imidacloprid resistance in *Myzus persicae* in Greece and susceptibility data for spirotetramat**. *Pest Management Science* pp. n/a–n/a.

Chapter 1

Drosophila suzukii (Diptera:
Drosophilidae) arrives at Minas
Gerais state, a main strawberry
production region in Brazil

Andreazza *et al* 2016. *Florida Entomologist*, 99(4):796-798



***Drosophila suzukii* (Diptera: Drosophilidae) Arrives at Minas Gerais State, a Main Strawberry Production Region in Brazil**

Author(s): Felipe Andrezza, Khalid Haddi, Eugenio E. Oliveira and João Alfredo M. Ferreira

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Drosophila suzukii (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil

Felipe Andreazza^{1,*}, Khalid Haddi², Eugenio E. Oliveira¹, and João Alfredo M. Ferreira^{1,3,*}

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), has recently invaded many countries and harmed soft-skinned fruit production worldwide (Dreves et al. 2009; Malsh et al. 2011; Burrack et al. 2013; Cini et al. 2014). In Brazil, *D. suzukii* was first recorded by Deprá et al. (2014) in a drosophilid diversity survey carried out in the southern regions of Brazil in 2013. In these regions, many of the preferred hosts are cultivated, but this pest has

been found infesting not only the traditional hosts such as strawberry (*Fragaria ananassa* Duch; Rosaceae) (Santos 2014) but also native fruits, such as Cattley guava (*Psidium cattleyanum* Sabine; Myrtaceae) and Surinam cherry (*Eugenia uniflora* L.; Myrtaceae) (Andreazza et al. 2015). Previously, however, heavy damages and economic losses have been reported only in strawberry production in the state of Rio Grande do Sul (Santos 2014).

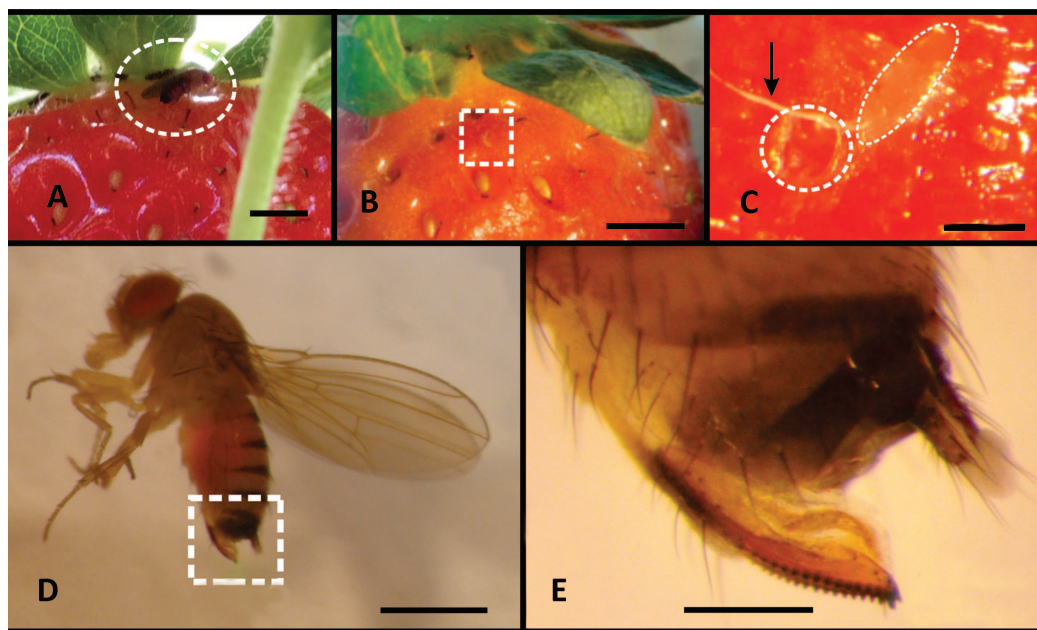


Fig. 1. *Drosophila suzukii* adult female collected at Minas Gerais State, Brazil. (A) Female on a strawberry fruit in the field, scale bar = 3 mm; (B) *D. suzukii* egg laid inside the fruit (white box), scale bar = 5 mm; (C) oviposition hole (white circle) with the egg's spiracles (black arrow) coming out. The egg laid beneath the fruit epidermis is delimited by a white ellipse, scale bar = 500 μm; (D) the female from image A viewed under microscope, scale bar = 500 μm; and (E) the female characteristic, serrated ovipositor, scale bar = 200 μm.

Universidade Federal de Viçosa, Departamento de Entomologia, 36.570-900, Viçosa–Minas Gerais, Brazil; E-mail: felipe.andreazza@ufv.br (F. A.),

khalid.haddi@ufv.br (K. H.), eugenio@ufv.br (E. E. O.), joao.marinho@ufv.br (J. A. M. F)

Science Without Borders, Universidade Federal de Viçosa, Departamento de Entomologia, 36.570-900, Viçosa–Minas Gerais, Brazil

Econtrole Pesquisa e Consultoria Ltda., CenTev/EBT, Caixa Postal 295, 36.570-900, Viçosa–Minas Gerais, Brazil

*Corresponding authors; E-mail: felipe.andreazza@ufv.br (F. A.), joao.marinho@ufv.br (J. A. M. F)

Minas Gerais is the biggest strawberry producer in Brazil, with around 1,700 ha in cultivation (IBGE 2014). As in Rio Grande do Sul, where *D. suzukii* was first observed causing economic losses in strawberry production (Santos 2014), arrival of this fly in Minas Gerais may cause enormous impact and affect both local growers and the main markets. *Drosophila suzukii* has spread throughout the Brazilian territory since its discovery in the states of São Paulo (Vilela & Mori 2014), Rio de Janeiro (Bitner-Mathé et al. 2014), and Goiás (Paula et al. 2014). Following reports from producers of high rates of unmarketable flaccid-like strawberries, the authors visited strawberry fields during Mar 2016 and surveyed for this pest.

In an organic production field located in the municipality of Ervália (20.8423083°S, 42.6756277°W), Minas Gerais, drosophilid adults were seen flying around both damaged and ripe, undamaged strawberries. Flies were collected using a handheld aspirator. Seven drosophilid flies were collected from damaged fruits and 1 fly was collected from undamaged fruit (Fig. 1A). One *D. suzukii* male was also seen (Fig. 2), but we failed to collect it. Undamaged fruit was collected to evaluate for the presence of *D. suzukii* eggs.

In the same field, 53 strawberry fruits presenting advanced flaccid-like symptoms (damaged) were collected, placed inside a plastic container (1.5 L) with vented openings, and returned to the laboratory in a Styrofoam cooler. Additionally, a guava tree (*Psidium guajava* L.; Myrtaceae) adjacent to the strawberry field showed symptoms of infestations by several pests, and 1 guava fruit was collected and returned to the laboratory.

Flies were examined under a stereomicroscope (at 40x) (SZX-SD02, Olympus Corporation, Tokyo, Japan). The strawberry and guava fruits were separately placed on a vermiculite layer inside 2 plastic cages containing venting openings sealed with voile cloth. Cages were checked daily, and the emerged flies were examined under the stereomicroscope and species determined using Vlach (2013); characters used in diagnostics, such as ovipositor (Fig. 1E) and wing pattern (Fig. 2) are illustrated.

One of the 7 flies collected from damaged fruit and 1 fly collected from the undamaged fruit were identified as females of *D. suzukii* (Fig. 1D). Furthermore, one *D. suzukii* egg was found inside the field-collected undamaged strawberry (Fig. 1B and C), and an adult *D. suzukii* male emerged 10 d later. From the damaged strawberry fruits, 32 *D. suzukii* adults (18 females and 14 males) emerged. As expected, other species of opportunistic secondary pests emerged from the fruits, primarily *Zaprionus indianus* Gupta (Diptera: Drosophilidae) (94 specimens), also seen in the field (Fig. 2), which is widely distributed in Brazil and Central and North America regions (Joshi et al. 2014; Van Timmeren & Isaacs 2014; Andreazza et al. 2015; Bernardi et al. 2015; Lasa & Tadeo 2015). From the guava fruit, 28 *Z. indianus* and 3 *D. suzukii* adults (2 females and 1 male) emerged, confirming that this native fruit is a suitable host (Andreazza et al. 2015).

The authors highlight here the importance of documenting *D. suzukii* in Minas Gerais, because this region is the main producer of strawberries in Brazil (IBGE 2014; Silveira & Guimarães 2014). Considering the potential of this species to infest other native hosts, additional work should be done to document the geographic and host range in Minas Gerais. As most management strategies for this pest are based on synthetic insecticides (Van Timmeren & Isaacs 2013), it is particularly important to develop and implement control strategies that are compatible with fruit production in this region and that can be a part of integrated pest management systems.

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Fig. 2. Damaged strawberry in the field with drosophilid flies including a *Drosophila suzukii* male (inlet) with its characteristic wing black dots (arrows) and 2 adults of *Zaprionus indianus* (white circles), scale bar = 5 mm.

Summary

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) was first collected in Minas Gerais State, Brazil, in Mar 2016, in the municipality of Ervália, from an organic strawberry field. In Brazil, this pest was first recorded in the southernmost region, in Rio Grande do Sul and Santa Catarina states. *Drosophila suzukii*'s arrival to Minas Gerais State, about 1,500 km north from its first record, should alarm the growers, the research community, and the authorities, because this region is the main strawberry production region in Brazil and is now susceptible to large increases in production losses caused by this invasive species. The lack of alternative effective management tools for *D. suzukii*, besides traditional chemical sprays, makes this pest an important area of study. Future research should focus on finding strategies that match with different local growing systems and edaphoclimatic conditions.

Key Words: spotted wing drosophila; first record; invasive pest; crop protection

Sumário

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) foi primeiramente coletada no estado de Minas Gerais em Março de 2016, no município de Ervália, em uma produção orgânica de morangos. No Brasil, esta praga foi primeiramente encontrada na região do extremo sul, nos estados do Rio Grande do Sul e Santa Catarina. A chegada de *D. suzukii* à Minas Gerais, aproximadamente 1.500 km ao norte do seu primeiro

registro, deve alertar os produtores, a comunidade científica e autoridades, já que esta região é a principal região produtora de morangos do Brasil e agora está suscetível à aumentos nas perdas da produção causadas por essa espécie invasiva. A falta de ferramentas de manejo efetivas alternativas para *D. suzukii*, além do controle químico tradicional, torna esta praga um importante objeto de estudo. Pesquisas futuras deverão focar em encontrar ferramentas que combinem com diferentes sistemas de produção e condições edafoclimáticas locais.

Palavras Chave: Drosófila-da-asa-manchada; primeiro relato; praga invasiva; fitossanidade

References Cited

- Andreazza F, Bernardi D, Botton M, Nava DE. 2015. Índice de infestação natural de *Drosophila suzukii* e *Zaprionus indianus* (Diptera: Drosophilidae) em frutíferas nativas no município de Pelotas, 4p. In XXIV Congresso de Iniciação Científica e XVII Encontro da Pós-Graduação, Pelotas. Anais. Universidade Federal de Pelotas, Pelotas, Brazil.
- Bernardi D, Andreazza F, Nava DE, Baronio CA, Botton M. 2015. Duplo Ataque. Cultivar HF 95: 16–19.
- Bitner-Mathé BC, Victorino J, Faria FS. 2014. *Drosophila suzukii* has been found in tropical Atlantic rainforest in southeastern Brazil. *Drosophila Information Service* 97: 136–137.
- Burrack HJ, Fernandez GE, Spivey T, Kraus DA. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), an invasive frugivore. *Pest Management Science* 69: 1173–1180.
- Cini A, Anfora G, Escudero-Colomar LA, Grassi A, Santosuosso U, Seljak G, Papini A. 2014. Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe. *Journal of Pest Science* 87: 559–566.
- 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.
- Dreves AJ, Walton V, Fisher G. 2009. A new pest attacking healthy ripening fruit in Oregon. Spotted wing drosophila: *Drosophila suzukii* (Matsumura). Oregon State University, Extension Service EM 8991.
- IBGE (Instituto Brasileiro de Geografia e Estatística). 2014. Censo Agropecuário, <http://www.sidra.ibge.gov.br/bda/tabela/protabl1.asp?t=819&z=p&o=2&i=P> (last accessed 6 Apr 2016).
- Joshi NK, Biddinger DJ, Demchak K, Deppen A. 2014. First report of *Zaprionus indianus* (Diptera: Drosophilidae) in commercial fruits and vegetables in Pennsylvania. *Journal of Insect Science* 14: 259–263.
- Lasa R, Tadeo E. 2015. Invasive drosophilid pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. *Florida Entomologist* 98: 987–988.
- Paula MA, Lopes PHS, Tidon R. 2014. First record of *Drosophila suzukii* in the Brazilian savanna. *Drosophila Information Service* 97: 113–115.
- Santos RSS. 2014. Ocorrência de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morango no Brasil. Embrapa Uva e Vinho, Bento Gonçalves, Brazil. (Comunicado Técnico 159).
- Silveira GRS, Guimarães BC. 2014. Aspectos sociais e econômicos da cultura do morangueiro. *Informe Agropecuário* 35: 7–10.
- Van Timmeren S, Isaacs R. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection systems. *Crop Protection* 54: 126–133.
- Van Timmeren S, Isaacs R. 2014. *Drosophila suzukii* in Michigan vineyards, and the first report of *Zaprionus indianus* from this region. *Journal of Applied Entomology* 138: 519–527.
- Vilela RC, Mori L. 2014. The invasive spotted wing drosophila (Diptera: Drosophilidae) has been found in the city of São Paulo (Brazil). *Revista Brasileira de Entomologia* 58: 371–375.
- Vlach J. 2013. Identifying *Drosophila suzukii*, <http://www.oregon.gov/oda/shared/documents/publications/ippm/spottedwingdrosophilaidkey.pdf> (last accessed 30 Mar 2016).
- Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee JC, Bruck J, Walton VM, O'Neal SD, Zalom FG. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management* 2: 1–7.

Chapter 2

Drosophilids infesting commercial soft berries in Espírito Santo State, Brazil

To be submitted to Journal of Insect Science, 2017

Drosophilids infesting commercial soft berries in Espírito Santo State, Brazil

Abstract

Invasion by an alien pest may greatly affect the economy and environment of a region. Such impacts can be reduced if the pest detection happens in an early stage of the invasion. *Drosophila suzukii* Matsumura is one of the most invasive insect-pest worldwide. This species lays eggs inside soft-skinned fruits, causing severe damage to these crops. *Zaprionus indianus* Gupta is another drosophilid fly capable of infesting some fruits and has been reported occurring along with *D. suzukii*. Thus, this note aimed to first report the occurrence of *D. suzukii* and *Z. indianus* infestation in blackberries and strawberries in the Espírito Santo state, Brazil. The cultivation of blackberries and strawberries is already limited by climate adequacy in this subtropical region, and the presence of *D. suzukii* and *Z. indianus* could further impact the establishment and production increases of this fruit species. Thus, our findings update the current *D. suzukii* expansion area within Neotropics. This will help to inform not only soft fruit producers, but also the authorities and the scientific community from this region, about the needs of actions to avoid *D. suzukii* spread and further potential damage to fruit production in these regions by the association of both reported species.

Keywords: Alien-pest, strawberry, blackberry.

2.1 Introduction

Identification of an alien pest invasion can result in savings of efforts, time and money. Thus, early field surveys monitoring the presence of a potential pest species are of importance, especially for the economically relevant hosts. *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is among the newest invasive insect pest in Neotropical regions (Deprá et al. 2014). It might be a result of the high dispersion abilities and broad host range of these flies (Asplen et al. 2015; Kenis et al. 2016). This insect pest is known as Spotted Wing Drosophila (SWD), and attacks several soft-skinned fruits such as blackberries (*Rubus* spp.), raspberries (*Rubus idaeus* L.), blueberries (*Vaccinium* spp.) and strawberries (*Fragaria* x *ananassa* Duchesne) (Bellamy et al. 2013). The larvae hatch from the eggs laid under the fruit epidermis and feed on the fruit tissues making the fruit unmarketable (Asplen et al. 2015). In Brazil, 30% of strawberry production loss was estimated under field conditions in Vacaria, Rio Grande do Sul state, in the first year of invasion of this pest (Santos 2014). Currently, *D. suzukii* has been reported from the south (Deprá et al. 2014) to the southeast Brazilian states, such as Rio de Janeiro (Bitner-Mathé et al. 2014) and Minas Gerais states (Andreazza et al. 2016b), but it had never been detected in Espírito Santo state.

Zaprionus indianus Gupta (Diptera: Drosophilidae) is another species that has been recently worldwide reported in SWD monitoring traps (Renkema et al. 2013; Joshi et al. 2014; Lasa and Tadeo 2015), as well as infesting fruits (Fartyal et al. 2014). In Brazil, this drosophilid was demonstrated to be able to attack undamaged strawberries in laboratory, with increasing oviposition abilities with previous SWD oviposition (Bernardi et al. 2017). Thus, even that *Z. indianus* is already present and dispersed throughout the Brazilian territory (Commar et al. 2012), the assessment of whether it is infesting berries prior harvest is of importance.

Espírito Santo state has a diverse climate range, which makes possible the cultivation of subtropical and tropical fruits (Incapér 2010), among which there are some main SWD and potentially *Z. indianus* hosts (i.e. strawberries and blackberries). Modeling predictions taking into account climate indexes has showed the invasion potential of SWD in Espírito Santo (Benito et al. 2016). Thus, the present work aimed to early detect, and report the presence and hosts of SWD in Espírito Santo state, Brazil,

and assess the infestation potential of *Z. indianus* in blackberry fields.

2.2 Material and Methods

Sampling to access *D. suzukii* and *Z. indianus* presence in Espírito Santo state was performed under field conditions in the highland region, particularly in Domingos Martins and Santa Maria de Jetibá, the two-major strawberry/black-berry-producers municipalities in the state (Incaper 2010). The first sampling was performed in July 8th, 2016 in Domingos Martins, at two strawberry fields (Figure 2.1) (a: 20°18'0.7"S, 41°04'5.8"W; and b: 20°23' 29.6"S, 41°01'55.3"W). A new and broader sampling were performed in February 21nd, 2017, at eight strawberry fields in both municipalities, and one blackberry field in Domingos Martins (Figure 2.1) [1: 20°22'35"S, 41°01'50"W; 2: 20°18'19"S, 41°01'46"W; 3: 20°15'15"S, 40°59'26.5"W; 4: 20°13'10.6"S, 40°59'6"W; 5: 20°08'19"S, 41°0'09"W; 6: 20°09'39"S, 40°55'09"W; 7: 20°10'28"S, 40°51'23.5"W; 8: 20°04'55.5"S, 40°53'25.5"W (strawberries) and 9: 20°22'18.6"S, 41°03'51.8"W (blackberries)].

Eventual presence of drosophilid adults was observed on fruits or in the canopy of plants in all fields sampled. These flies were collected with a hand aspirator and deposited in 96° GL alcohol for later identification. Additionally, 60 to 110 fruits were

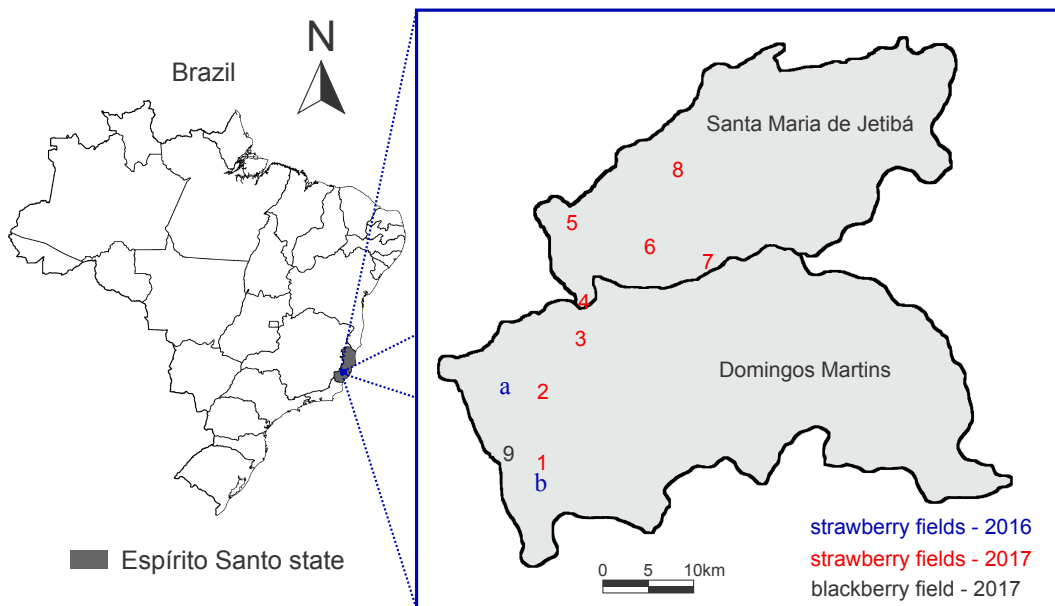


Figure 2.1: Blackberry and strawberry sampling sites in the municipalities of Domingos Martins and Santa Maria de Jetibá, Espírito Santo state, Brazil.

randomly collected from each field, and placed on a layer of paper towels inside plastic boxes (20x30x17 cm) with venting openings, closed with fine mesh, and sent to the Laboratory at the Universidade Federal de Viçosa, to verify adults' emergence. Emerged flies were daily collected along 14 days for later species identification. Key of Vilela and Mori (2014) was used to identify *D. suzukii* specimens, and *Z. indianus* was identified according to Van der Linde (2010). Other drosophilids also occurred but were not identified to species level.

2.3 Results and Discussion

Drosophila suzukii specimens were not present among the drosophilid adult flies collected in the first sampling year (i.e. 2016) under field conditions. *Drosophila* spp. were the most common species collected (n = 485) in the strawberry field, followed by *Z. indianus* (16). The first *D. suzukii* specimens found in Espírito Santo state (28 ♂ and 8 ♀) were collected as adults directly from the blackberry fruits in Domingos Martins, Brazil, in February 2017 (Figure 2.2). A total of 965 *D. suzukii* adults (sex ratio = 0.54), 458 *Z. indianus*, and 21 *Drosophila* spp. emerged from field collected blackberry fruits (n = 76), demonstrating a very high infestation by *D. suzukii* in this region with no previous report of this pest species. (Figure 2.3). *Drosophila suzukii* were found in six of the eight strawberry fields surveyed in the second year (Figure 2.4A). This species was only collected directly as adult from the strawberry canopy (1 ♀) in the field number 1. *Zaprionus indianus* and *Drosophila* spp. were present in all collected strawberry fruits, and at higher infestation (Figure 2.4B).

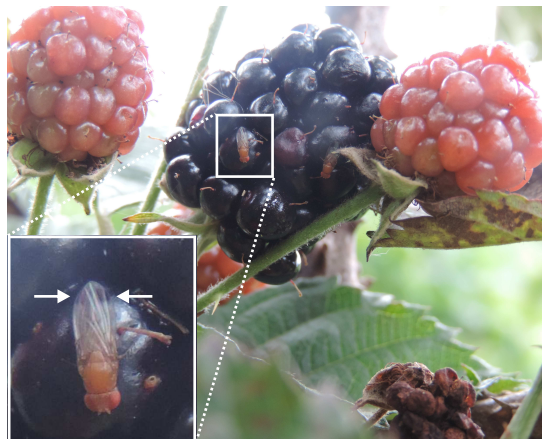


Figure 2.2: Blackberry fruits with *Drosophila suzukii* male flies. The white arrows (inlet) indicate the male's characteristic wing black-dots.

The results have showed that *D. suzukii* is present in the highlands of Espírito Santo state, and that high infestation was found in blackberry fruits, which is a well-known *D. suzukii* host (Bellamy et al. 2013; Burrack et al. 2013). On the other hand, a very low infestation level was recorded in strawberries (and only during the second survey year), despite its recognized relevance as *D. suzukii* host in Neotropical regions (Bernardi et al. 2017). This low infestation level can be a result of an initial stage of the invasion or a reflect of the advanced damage conditions of the sampled fruits. According to Keesey et al. (2015), *D. suzukii* flies seem to have shifted their preferential niche from damaged decaying to healthy undamaged ripe fruits and only visually damaged strawberry fruits were collected in the present study. The damage condition of the collected fruits may also explain the occurrence of high number of *Drosophila* spp. and *Z. indianus* emerging from strawberry (Figure 2.4B) while almost no emergence of this flies were obtained from the blackberry fruits (Figure 2.3). *Zaprionus indianus* was not only reported at high infestation here but they have also been consecutively reported under field conditions and traps in several other regions, such as in Asia and North America (Fartyal et al. 2014; Joshi et al. 2014; Lasa and Tadeo 2015) and were shown to be able of infesting health undamage strawberries in laboratorry assays (Bernardi et al. 2017). Thus, the infestation abilities of *Z. indianus* in undamaged or early *D. suzukii* damaged blackberries should be further investigated, both in laboratory and in more extensive field surveys.

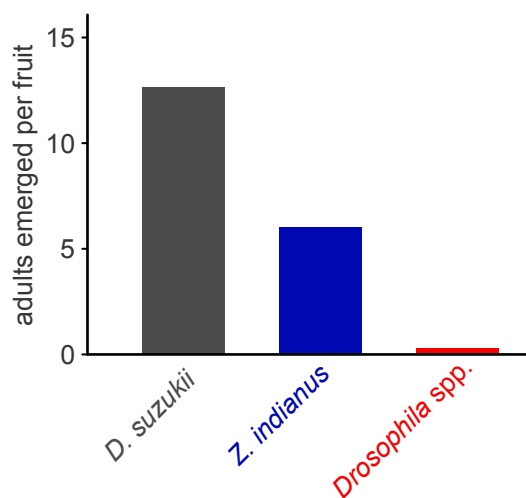


Figure 2.3: Number of drosophilid adults emerged from blackberries collected at 'Fazenda Experimental Mendes da Fonseca, Incaper', municipality of Domingos Martins, Espírito Santo, Brazil, in February 2017.

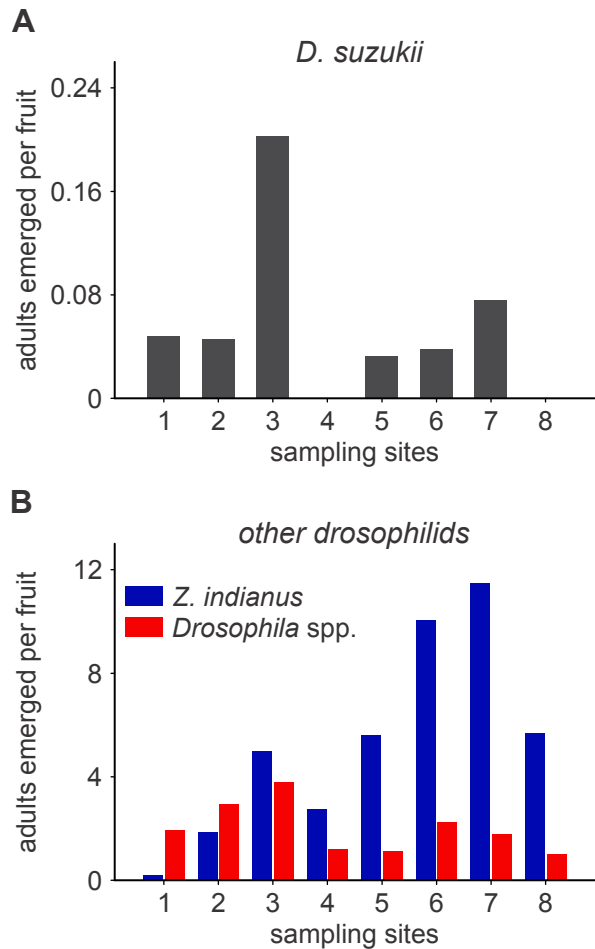


Figure 2.4: Number of *Drosophila suzukii* (A) and other drosophilid flies (B) emerged from strawberry fruits collected at eight different sites in the municipalities of Domingos Martins and Santa Maria de Jetibá, Espírito Santo, Brazil, in February 2017. Please note the scale difference in the y-axis between panels.

The report of *D. suzukii* in the Espírito Santo state should be an alert to local stakeholders, researchers, and extension services for new surveys aiming to avoid its spread throughout this highland region. These actions should reduce the economic impact of this pest invasion in this Brazilian state. In the region, other *D. suzukii* main hosts not evaluated in this work, such as grapes (*Vitis vinifera* L.), loquats [*Eriobotrya japonica* (Thunb.) Lindl.] and raspberries are also cultivated. Future surveys and researches might focus also in these fruit species. Since in the Espírito Santo state there is a shift from some subtropical to tropical fruit productions (e.g., papaya - *Carica papaya* L.), it might be worth to perform early laboratory susceptibility tests with these tropical fruit species and other potential new hosts. It will surely help to early predict the potential of further dispersion throughout this and other tropical Brazilian states.

Thus, it is concluded that *D. suzukii* has expanded its invasion area in Brazil, being

already present in the highlands of Espírito Santo state. This species was found in higher infestation in blackberries, however in early stage in strawberries. *Zaprionus indianus* was found in high infestation in both blackberries and strawberries, being this the first report of this drosophilid fly infesting visually undamaged blackberries before harvesting.

2.4 Acknowledgements

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References

- Andreazza F, Haddi K, Oliveira EE, Ferreira JAM (2016b) ***Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil.** *Florida Entomologist* 99:796–798.
- Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Kárpáti Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Vétek G, Vogt H, Walton VM, Yu Y, Zappalà L, Desneux N (2015) **Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities.** *Journal of Pest Science* 88:469–494.
- Bellamy DE, Sisterson MS, Walse SS (2013) **Quantifying host potentials: Indexing postharvest fresh fruits for Spotted Wing *Drosophila*, *Drosophila suzukii*.** *Plos ONE* 8:e61227.
- Benito NP, Lopes-da Silva M, Santos RSSd (2016) **Potential spread and economic impact of invasive *Drosophila suzukii* in Brazil.** *Pesquisa Agropecuária Brasileira* 51:571–578.
- Bernardi D, Andreazza F, Botton M, Baronio CA, Nava DE (2017) **Susceptibility and interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in damaging strawberry.** *Neotropical Entomology* 46:1–7.
- Bitner-Mathé BC, Victorino J, Faria FS (2014) ***Drosophila suzukii* has been found in tropical Atlantic Rainforest in southeastern Brazil.** *Drosophila Information Service* 97:136–137.
- Burrack HJ, Fernandez GE, Spivey T, Kraus DA (2013) **Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore.** *Pest Management Science* 69:1173–1180.

- Commar LS, Galego LGdC, Ceron CR, Carareto CMA (2012) **Taxonomic and evolutionary analysis of *Zaprionus indianus* and its colonization of Palearctic and Neotropical regions.** *Genetics and Molecular Biology* 35:395–406.
- 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.
- Fartyal RS, Sarswat M, Lhamo N, Sati P, Asha L (2014) **Records of *Zaprionus indianus* and *Drosophila suzukii* indicus as invasive fruit pests from mid valley region of Garhwal Uttarakhand, India.** *Drosophila Information Service* 97:119–123.
- Incaper (2010) **Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER): 10 anos de integração pesquisa-extensão.** *Incaper em Revista* 1:145.
- Joshi NK, Biddinger DJ, Demchak K, Deppen A (2014) **First report of *Zaprionus indianus* (Diptera: Drosophilidae) in commercial fruits and vegetables in Pennsylvania.** *Journal of Insect Science* 14:1–4.
- Keesey IW, Knaden M, Hansson BS (2015) **Olfactory specialization in *Drosophila suzukii* supports an ecological shift in host preference from rotten to fresh fruit.** *Journal of Chemical Ecology* 41:121–128.
- Kenis M, Tonina L, Eschen R, van der Sluis B, Sancassani M, Mori N, Haye T, Helsen H (2016) **Non-crop plants used as hosts by *Drosophila suzukii* in Europe.** *Journal of Pest Science* 89:735–748.
- Lasa R, Tadeo E (2015) **Invasive drosophilid pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico.** *Florida Entomologist* 98:987–988.
- Renkema J, Miller M, Fraser H, Légaré JP, Hallett R (2013) **First records of *Zaprionus indianus* Gupta (Diptera: Drosophilidae) from commercial fruit fields in Ontario and Quebec, Canada.** *Journal of the Entomological society of Ontario* 144:125–130.
- Santos RSS (2014) **Ocorrência de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morango no Brasil.** *Comunicado Técnico/ Embrapa Uva e Vinho* 159:4.
- Van der Linde K (2010) ***Zaprionus indianus*: species identification and taxonomic position.** *Drosophila Information Service* 93:95–98.
- Vilela CR, Mori L (2014) **The invasive spotted-wing *Drosophila* (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil).** *Revista Brasileira de Entomologia* 58:371–375.

Chapter 3

**Differential insecticide
susceptibility of *Drosophila suzukii*
(Diptera: Drosophilidae)
populations from Minas Gerais,
Brazil**

To be submitted to Journal of Pest Science, 2017

Differential insecticide susceptibility of *Drosophila suzukii* (Diptera: Drosophilidae) populations from Minas Gerais, Brazil

Abstract

The assessment of insecticide susceptibility is one of the first steps in order to better estimate the likelihood of control failure of invasive pests. *Drosophila suzukii* (Diptera: Drosophilidae) is among the most important invasive pest worldwide, and has recently (i.e. 2016) invaded Minas Gerais state, Brazil. Thus, the present investigation was conducted aiming to evaluate the susceptibility to commercial insecticides of *D. suzukii* populations originally collected in the Brazilian Minas Gerais state, and a *D. suzukii* population from Rio Grande do Sul state, Brazil, reared under laboratory conditions for 48 generations (and hereafter termed as insecticide susceptibility pattern). Firstly, concentration-mortality bioassays were conducted in order to estimate the lethal concentrations (LCs) of five selected insecticides (i.e., deltamethrin, permethrin, imidacloprid, indoxacarb and azadirachtin) to the insecticide susceptibility pattern population. After that, by applying discriminatory concentrations (i.e., LC₂₅, LC₅₀, and LC₉₀) of the insecticides that exhibit potential of being used as control tools, we could assess the susceptibility to these insecticides on the *D. suzukii* populations from Minas Gerais. Among the five tested products only the two pyrethroids (i.e., deltamethrin and permethrin) and the neonicotinoid imidacloprid were significantly toxic to *D. suzukii*. Our results demonstrated that when exposed to the imidacloprid LC₂₅ or LC₅₀, the population originally collected at Ervália - MG, was more susceptible ($P < 0.05$) than both the population originally collected at Juiz de Fora - MG and the insecticide susceptibility pattern population. The population originally collected at Juiz de Fora, however, was more resistant ($P < 0.05$) than the two other populations at all the imidacloprid concentrations tested (i.e., LC₂₅, LC₅₀ and LC₉₀). No consistent differences were found for permethrin. Thus, although still not registered to control *D. suzukii* in Brazil, the present investigation showed neonicotinoid resistance in some

Brazilian populations of these flies, which may compromise the use these molecules as potential tool to control *D. suzukii* infestations in Brazil.

Keywords: resistance, toxicity, Spotted Wing Drosophila, neonicotinoid, imidacloprid.

3.1 Introduction

Highly invasive pests have been a topic of concern in every invaded area, especially when it comes together with the indiscriminate use of chemicals as an emergency control strategy. The use of insecticides are known to cause selection of resistant populations (Devonshire et al. 1998; Daborn et al. 2001; Campos et al. 2014; Sparks and Nauen 2015; Guedes 2017). Therefore, studies aiming to evaluate the differential susceptibility to insecticides of different populations of an invasive species are essential to support decisions on rotation of chemical classes (Croft 1990; Sparks and Nauen 2015), avoiding future control failures. Even so that studies from old invaded regions certainly provide a fair idea of insecticides efficacies, the genetic diversity of the species from the continental or local newly invaded regions should be considered (Adrion et al. 2014). Thus, toxicological studies on different local populations of the pest species are important to early detect resistant sources (Campos et al. 2014; Roiditakis et al. 2015; Silva et al. 2015). This early monitoring can help to prevent field control failures (Guedes 2017), and the set up of management strategies against insecticide resistance selection (Croft 1990; Mishra et al. 2017).

Among the newly invasive pest worldwide, *Drosophila suzukii* (Diptera: Drosophilidae) possesses biological characteristics such as short generation time and high reproductive outputs (Emiljanowicz et al. 2014; Tochen et al. 2014) associated with very high polyphagia (Kenis et al. 2016) making it a very difficult pest to be managed. Thus, despite several alternative control strategies have being made available to manage this pest (Hampton et al. 2014; Daane et al. 2016; Guerrieri et al. 2016), the spray of insecticides to mitigate its losses is still very common (Haye et al. 2016). The zero-tolerance politics for the pest presence of some regions (Van Timmeren and Isaacs 2013), further increases the broad-spectrum insecticide application against this pest, raising even more the possibilities of resistance selection, highlighting the needs for more insecti-

cide resistance monitoring studies (Mishra et al. 2017).

To our knowledge, neither high resistance was reported nor laboratory selection has been successful in simulating this phenomenon for *D. suzukii* (Smirle et al. 2017). However, a recent study found low resistance ratios of *D. suzukii* to 3 broad-spectrum insecticides (i.e. zeta-cypermethrin, spinosad, and malathion) (Mishra et al. 2017). These authors reveal that no control failure would be expected from these difference levels, but they demonstrate the complexity and diversity of detoxification gene expressions induced by the insecticide exposition that represents a highly diverse resistance source in *D. suzukii*. As well as is expected for *D. suzukii*, cases of insecticide resistance in other drosophilid flies, as *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Pittendrigh et al. 1997; Daborn et al. 2001; Bogwitz et al. 2005; Perry et al. 2008), or in other dipterans (Zhong et al. 2013; Haddi et al. 2017) are not uncommon.

Several studies have looked at the efficacy of field doses rate to kill *D. suzukii* (Bruck et al. 2011; Van Timmeren and Isaacs 2013; Andreatza et al. 2017), but only few have determined the full toxicity range [i.e. lethal concentration (LC) or lethal doses (LD)] of some insecticides (Hamby et al. 2013; Jang et al. 2017; Mishra et al. 2017; Smirle et al. 2017). In Brazil, where the species has being present for about 4 years (Deprá et al. 2014), there are neither insecticides registered to be used against *D. suzukii* (MAPA 2017), nor information about full range toxicity or differential susceptibility to insecticides of local populations of this pest. However the high field selection pressure imposed by emergency control in older invaded regions (i.e. North America and Europe) (Beers et al. 2011; Bruck et al. 2011; Haviland and Beers 2012; Van Timmeren and Isaacs 2013), which might be the origin of the *D. suzukii* invasion in South America (Deprá et al. 2014), increases the possibility that resistance has been already selected in some populations before its arrival to the South America territory.

Thus, in the current study we aimed to assess the differential susceptibility to commercial insecticides in *D. suzukii* populations originally collected in the Brazilian Minas Gerais state, and in a reference population that were maintained in laboratory culture for about 48 generations. The results from this study provide substantive information of potential resistant sources in this species, a still overlooked area.

3.2 Material and Methods

3.2.1 Insects rearing and chemicals

All bioassays and insects rearing were conducted in the same laboratory condition (25 ± 2 °C, RH of 50 ± 10 and 12 h of photophase). The fly populations were originally collected as larvae in infested fruits from the field, at different sites (Table 3.1) and were maintained in the laboratory on artificial diet (Emiljanowicz et al. 2014; Andreazza et al. 2016a) until bioassays were performed. The *D. suzukii* population that were originally collected from the field in Pelotas-RS, Brazil (Table 3.1), in September of 2014, were used in the concentration-mortality bioassays and as the “reference” population in the susceptibility bioassays, and hereafter is termed as insecticide susceptibility pattern. The selected chemicals used in this study were obtained from local market as formulated commercial insecticides (Table 3.2).

3.2.2 Concentration-mortality bioassays

The exposure procedures followed the IRAC protocol No: 026, recommended for bioassays with *Musca domestica* L. (Diptera: Muscidae) adults, with modifications (IRAC 2011). Each sample unit consisted of a 200 mL glass jar containing a 2 cm long dental cotton wick impregnated with 1.9 mL of the chemical solution at the desired

Table 3.1: *Drosophila suzukii* populations sources and the number of generations under laboratory culture before the bioassays to be performed.

Population	Collecting site (municipality)	Coordinates	High	Host	Generations ^a
RS	Pelotas	31°37'23.98"S	166 m	loquat	48-50 ^b
	RS - Brazil	52°31'21.61"W			64-65 ^c
ER	Ervália	20°50'32.31"S	753 m	strawberry	19 - 20 ^c
	MG - Brazil	42°40'32.26"W			
JF	Juiz de Fora	21°45'04.71"S	754 m	strawberry	4 - 5 ^c
	MG - Brazil	43°22'23.70"W			

^aNumber of generations under laboratory culture before the concentration-response (^b) or susceptibility (^c) bioassays were performed.

concentration. The top of each jar was sealed with a foam plug. Inside the jar, were released 20 to 25 3-4 days old flies randomly collected from the rearing cage with the aid of a hand aspirator. After 24 h, the flies' mortality was visually assessed. It was considered as dead the flies that did not show any movement even after stimulation with a fine brush. All the chemicals were diluted in a solution of water with 20% of sugar (m/v), which consisted also the solution used as control treatment.

Concentration-response mortality was performed using the insecticide susceptibility pattern population. For that, pre-tests were performed using 4 replicates for each one of 4 to 5 logarithmic spaced concentrations of the selected chemicals, to determine the concentration range in which each chemical starts to cause mortalities between 0 and 100% of the exposed flies. Using 6 to 7 concentrations within the determined ranges, the exposition bioassays were repeated, with 4 replicates per concentration, and the mortality data were then used to estimate the lethal concentration (LC) curves for each chemical.

3.2.3 Comparative susceptibility bioassays

To assess the insecticide susceptibility differences among the *D. suzukii* populations originally collected in Minas Gerais state (MG), Brazil and the insecticide susceptibility pattern population (Table 3.1), adult flies were simultaneously exposed to the concentrations of permethrin and imidacloprid, estimated to kill 25, 50 and 90% (LC₂₅, 50 and 90) of the exposed flies in the previous bioassay. Four replicates were used in each

Table 3.2: Formulated insecticides used in the toxicity and susceptibility bioassays with *Drosophila suzukii* adults.

Active ingredient (concentration)	Commercial name	Manufacturer	Chemical class
deltamethrin (25 g L ⁻¹)	Decis®	Bayer Cropscience Co.	type II pyrethroid
permethrin (250 g L ⁻¹)	Talcord®	BASF Co.	type I pyrethroid
imidacloprid (700 g kg ⁻¹)	Evidence®	Bayer Cropscience Co.	neonicotinoid
azadirachtin (12 g L ⁻¹)	Azamax®	E.I.D. Parry Limited	tetranotriterpenoids
indoxacarb (300 g Kg ⁻¹)	Rumo®	DuPont S.A.	oxadiazine

concentration for each population for both permethrin and imidacloprid insecticides. The mortality were assessed at 24h following the same protocol than the previous bioassays.

3.2.4 Data analysis

The mortality data of the concentration response bioassays were submitted to a probit analysis [PROC PROBIT, SAS Institute, (2013)]. The toxicity ratios among the four selected insecticide were calculated according to Robertson et al. (2007). After checking the assumptions of normality by Shapiro-Wilk and the equality of variances, the mortality data of the comparative susceptibility bioassays were submitted to an ANOVA followed by a Post Hoc Multiple Comparisons Tukey test on Sigma Plot 12.5 (Systat Software, San Jose, CA) ($P < 0.05$).

3.3 Results

3.3.1 Concentration-response bioassays

Except from indoxacarb and azadirachtin, the observed data for all the products on the insecticide susceptibility pattern population fit in the probit analysis with low χ^2 ($P > 0.05$) (Figure 3.1) (Table 3.3). The tested concentrations for deltamethrin varied from 0.5 to 8 mg of a.i. L⁻¹. For permethrin this variation was from 100 to 900 mg of a.i. L⁻¹ and for imidacloprid it varied from 20 to 1100 mg of a.i. L⁻¹. The LC curve and its 95% confidence interval for each product are displayed at the Figure 3.1. For azadirachtin, concentrations as high as 4800 mg of a.i. L⁻¹ resulted in low mortalities (i.e. < 50 %). For indoxacarb the scenario was even worst, being all concentrations as high as 10000 mg of a.i. L⁻¹ resulting in mortalities lower than 25%. Therefore, azadirachtin and indoxacarb were not considered in the rest of the study. Considering the deltamethrin insecticide as the reference for toxicity comparisons at LC₅₀ on the insecticide susceptibility pattern population, the imidacloprid was the second more toxic [Toxicity ratio (TR) = 34.7], followed by permethrin (TR = 49.3) (Table 3.3).

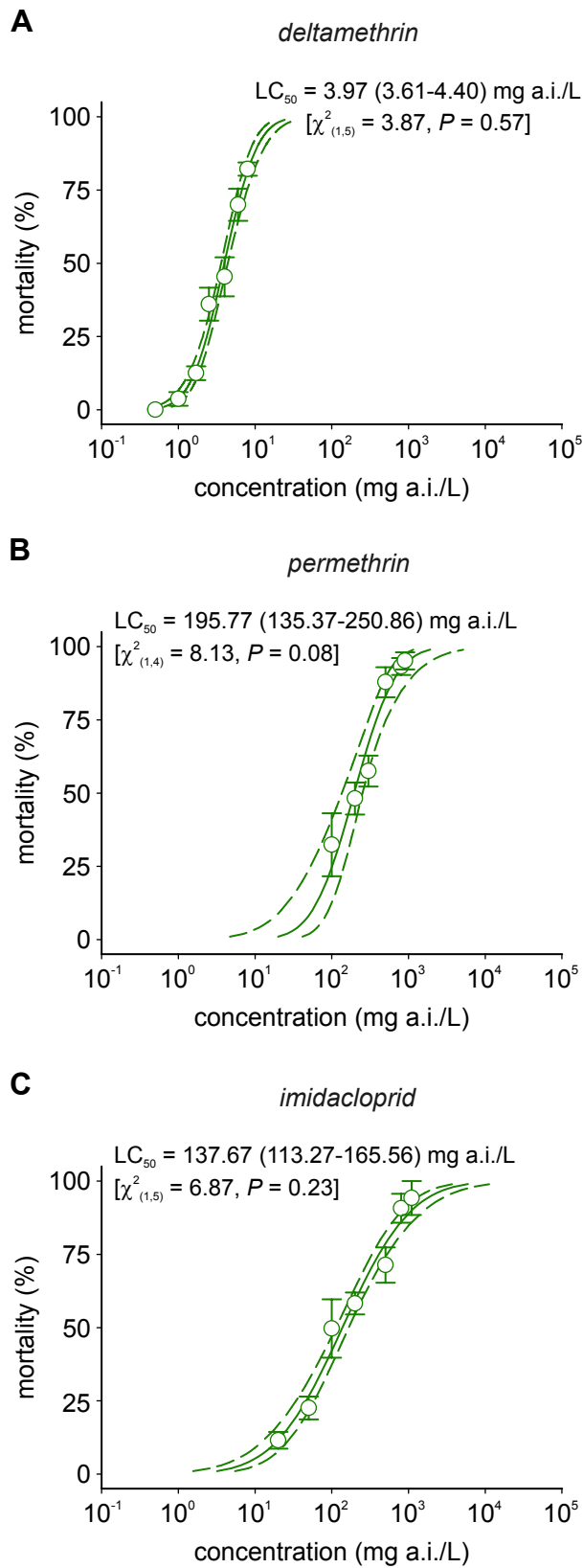


Figure 3.1: Concentration-response curves of three synthetic insecticides (continuous lines) with their 95% confidence intervals (dashed lines) from the Probit analysis on the *Drosophila suzukii* insecticide susceptibility pattern population.

Table 3.3: Tolerance of *Drosophila suzukii* Lab. population to selected insecticides and the insecticides toxicity ratio on the adults flies.

Chemical	<i>n</i>	LC ₅₀ (95% CI) ^a	slope ± SE	χ ²	<i>df</i>	TR (95% CI) ^b
deltamethrin	608	3.97 (3.61 - 4.40)	2.95 ± 0.23	3.87	5	1 (0.9 - 1.1)
permethrin	595	195.77 (135.37 - 250.86)	2.35 ± 0.29	8.13	4	49.3 (40.3 - 60.3)
imidacloprid	604	137.67 (113.27 - 165.56)	1.42 ± 0.11	6.87	5	34.7 (24.2 - 49.6)

^a Lethal concentration estimated to kill 50% of the exposed insects in mg of a.i. L⁻¹ with its respective confidence intervals at 95% of probability level. ^b Toxicity ratio (LC₅₀ of insecticide "X" / LC₅₀ of deltamethrin) with its respective confidence intervals at 95% of probability level. If the CI does not include the 1.0 the TR are significantly different.

3.3.2 Comparative susceptibility bioassays

The susceptibility comparison among the two populations from MG state (ER: Ervália and JF: Juiz de Fora) with the insecticide susceptibility pattern population, demonstrated differences ($P < 0.05$) in susceptibility mainly for imidacloprid, at the three tested concentrations (LC₂₅, 50 and 90) (Figure 3.2). The percentage of mortality for the ER population was higher than for the insecticide susceptibility pattern and JF populations after exposure to both the LC₂₅ ($F_{2,9} = 54.20, P < 0.001$) and LC₅₀ ($F_{2,17} = 124.95, P < 0.001$) of imidacloprid (Figure 3.2B). The JF population, on the other hand, was more resistant to imidacloprid. Its mortality percentages were lower than the insecticide susceptibility pattern and ER populations after exposure to both the LC₂₅ ($F_{2,9} = 54.20, P < 0.001$), LC₅₀ ($F_{2,17} = 124.95, P < 0.001$) and LC₉₀ ($F_{2,8} = 43.30, P < 0.001$) of imidacloprid (Figure 3.2B). For permethrin, however, there was difference among the populations only at the LC₅₀ ($H = 7.45, df = 2, P = 0.013$) but not at the LC₂₅ ($F_{2,9} = 0.16, P = 0.855$) and LC₉₀ ($F_{2,9} = 0.99, P = 0.408$) (Figure 3.2A).

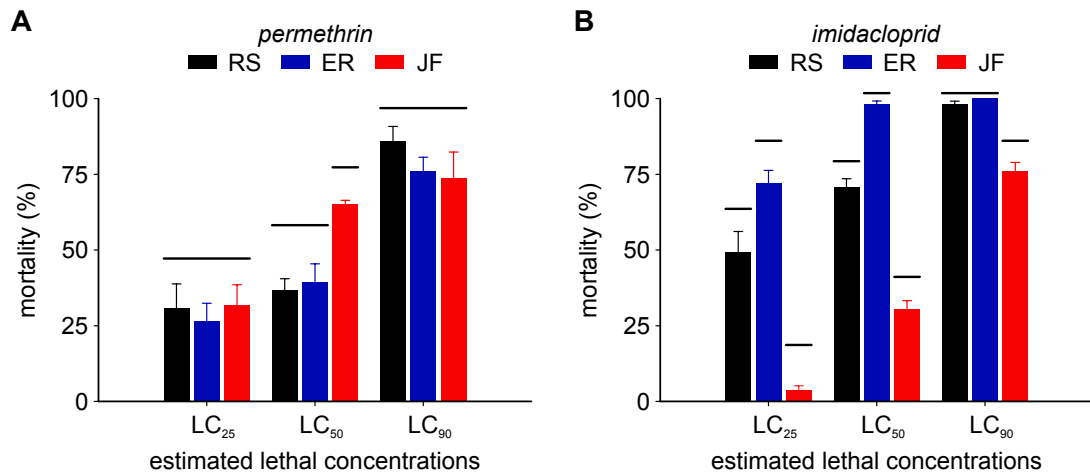


Figure 3.2: Susceptibility comparisons [mortality (mean \pm SE)] of *Drosophila suzukii* adults from different populations at three estimated lethal concentrations (LC) of permethrin (A) and imidacloprid (B). The LC values were estimated on bioassays with the insecticide susceptibility pattern (RS). Vertical bars under the same horizontal diagram do not differ from each other ($P > 0.05$). Note: RS = insecticide susceptibility pattern population; ER = population originally collected at Ervália - MG, Brazil; JF = population originally collected at Juiz de Fora - MG, Brazil.

3.4 Discussion

Insecticide resistance selection and control failures are an undesired common phenomenon occurring with several pest species (Araújo et al. 2011; Haddi et al. 2012; Guedes 2017; Haddi et al. 2017). Here, in addition to show differential toxicities of three selected insecticides on *D. suzukii* adults, we report a first insight of a natural resistance selection to a neonicotinoid insecticide (i.e. imidacloprid) in a population of *D. suzukii*. Furthermore, currently, all toxicological information provided for any South American *D. suzukii* population is still based only on discriminatory field rate doses already established for other pest species (Andreazza et al. 2017). Thus, the results reported here, assessing the full range of lethal concentrations of different compounds to a local insecticide susceptibility pattern population, and comparing the insecticide susceptibility of different populations are of extreme importance in helping the set up of management strategies against resistance selection and control failures (Croft 1990; Mishra et al. 2017).

Neonicotinoid resistance is reported in several pest species to be a result of mutations in the acetylcholine receptors (Liu et al. 2006; Perry et al. 2008; Bass et al. 2011) or over-expression of detoxification enzymes, especially of the P450 family (Daborn

et al. 2001; Elzaki et al. 2017; Kaplanoglu et al. 2017), as was demonstrated to occur for *D. suzukii* exposed to other chemicals groups (Mishra et al. 2017). Thus, further laboratory-based pressure selection on the JF population should be performed to foresee a field resistance selection to imidacloprid and allow a fully characterization of the possible neonicotinoid resistance mechanisms occurring in *D. suzukii*. Such studies could end up creating more advanced monitoring techniques (e.g. TaqMan Real-Time PCR) (Haddi et al. 2012) that allow rapid measurement of field frequencies of resistant specimens (Silva et al. 2015) coupled with large compressive sample approaches (Guedes 2017).

The potential for a fast insecticide selection in *D. suzukii* is indicated by the current results, which shows that within only one year from the first report of this invasive pest in the sampled region (Andreazza et al. 2016b), there are already differences in susceptibility to imidacloprid between two potentially closely related populations (originally collected 125 km from each other). Currently, there are no insecticides registered within the government controlling agencies to be used against *D. suzukii* in Brazil, but applications with neonicotinoids (i.e., imidacloprid, acetamiprid and thiamethoxam) on some *D. suzukii* main hosts, as strawberries, guavas, peaches and grapes are allowed (MAPA 2017). This may explain the differences here found. Nevertheless, worldwide, even that less toxic than pyrethroids and organophosphates, neonicotinoids are commonly used in rotations to manage *D. suzukii* infestations (Van Timmeren and Isaacs 2013; Bruck et al. 2011).

The chemical control still remains as the main used tool to manage *D. suzukii* in most of the invaded areas (Asplen et al. 2015; Haye et al. 2016). Therefore, this panorama, together with some biological characteristics of the species (Emiljanowicz et al. 2014; Tochen et al. 2014), further explain the possibility of a fast selection of a insecticide resistant population. The rapid selection of insecticide resistance as a compound result of both the pest biology, the management strategies and the naturally occurring high number/frequency of resistance sources is not uncommon (Campos et al. 2014; Reditakis et al. 2015; Kaplanoglu et al. 2017). *Drosophila suzukii* from two field location in USA was recently demonstrated to differentially express a complex high number of detoxification genes as well as having variable numbers of Single Nucleotide Variant mutations, indicating a high resistance source in this species (Mishra et al. 2017). A

high number of resistance sources, from target site mutations (Pittendrigh et al. 1997; Perry et al. 2008) to detoxification (Daborn et al. 2001; Bogwitz et al. 2005) also occurs in other drosophilid species studied worldwide.

Considering only populations from South American region, full information on the lethal concentrations is provided only for the three selected insecticide in the current study. Among these, the pyrethroid ones (i.e. deltamethrin and permethrin) shared the higher toxicities with imidacloprid. Deltamethrin was de most toxic showing the lowest values of LC_{50} , and therefore, locally, it could be effectively used in rotation with other products to broke any possible selection cycle to imidacloprid. The same is valid for permethrin, since both populations originally collected from MG state did not present differences in susceptibility to this insecticide. Malathion, which is also effective against *D. suzukii* populations worldwide (Bruck et al. 2011; Andreatza et al. 2017; Smirle et al. 2017), did not successfully select a resistant population in laboratory selection bioassays with a population from Canada (Smirle et al. 2017). Thus, this insecticide could also be potentially considered in rotation with other chemical classes. Nevertheless, the current results, even that combined with other studies [e.g. Smirle et al. (2017)], certainly does not provide a full panorama of the insecticides resistance sources and its field frequencies in this species. For that, surveys covering larger areas, and continuous insecticide resistance monitoring studies with a more comprehensive spatial sampling (Guedes 2017) are needed in order to mitigate the risks of resistance selection and control failure in this species. Finally since currently there are no insecticides registered to be used against *D. suzukii* in Brazil (MAPA 2017), no field recommendations can be formally made for any Brazilian territory.

The results presented here contribute to the knowledge of the toxicities of three selected insecticides on an insecticide susceptibility pattern population that was originally collected from the South American first invaded area (i.e. southern Brazil) approximately two years after its invasion. This information can serve as comparison basis for future studies monitoring the evolution of field populations' susceptibilities. The difference in susceptibility to imidacloprid found among *D. suzukii* populations from MG state in the current study does not necessarily represent occurrence of a resistant population in the field, but it does indicate the potential source of neonicotinoid resistance mechanisms in Braziliam populations. Thus the further laboratory selection

suggested early in the discussion, and more field surveys are surely necessary to avoid future control failures if ever a neonicotinoid be registered to be used against *D. suzukii* in Brazil. Since no information on potential neonicotinoid resistance in *D. suzukii* from other invaded regions (e.g. North America and Europe) are still reported, the current worldwide recommendation for an area-wide integrated pest management (Asplen et al. 2015; Haye et al. 2016), with special attention to the rotation of efficient insecticides covering different actions modes (Sparks and Nauen 2015) is naturally recommended for the countries there are registered products against *D. suzukii*.

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References

- Adrion JR, Kousathanas A, Pascual M, Burrack HJ, Haddad NM, Bergland AO, Machado H, Sackton TB, Schlenke TA, Watada M, Wegmann D, Singh ND (2014) ***Drosophila suzukii*: The genetic footprint of a recent, worldwide invasion.** *Molecular Biology and Evolution* 31:3148–3163.
- Andreazza F, Bernardi D, Baronio CA, Pasinato J, Nava DE, Botton M (2017) **Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae).** *Pest Management Science* 73:146–152.
- Andreazza F, Bernardi D, Marangon RB, Scheunemann T, Botton M, Nava DE (2016a) **Técnica de criação de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) em dieta artificial.** *Boletim de Pesquisa e Desenvolvimento/Embrapa Clima Temperado* 240:23p.
- Andreazza F, Haddi K, Oliveira EE, Ferreira JAM (2016b) ***Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil.** *Florida Entomologist* 99:796–798.
- Araújo RA, Williamson MS, Bass C, Field LM, Duce IR (2011) **Pyrethroid resistance in *Sitophilus zeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel.** *Insect Molecular Biology* 20:437–445.
- Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Kárpáti Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Véték G, Vogt H, Walton VM, Yu Y, Zappalà L, Desneux N (2015) **Invasion biology of spotted wing *Drosophila***

- (*Drosophila suzukii*): a global perspective and future priorities.** *Journal of Pest Science* 88:469–494.
- Bass C, Puinean AM, Andrews M, Cutler P, Daniels M, Elias J, Paul VL, Crossthwaite AJ, Denholm I, Field LM, Foster SP, Lind R, Williamson MS, Slater R (2011) **Mutation of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*.** *BMC Neuroscience* 12:1471–2202.
- Beers EH, Van Steenwyk RA, Shearer PW, Coates WW, Grant JA (2011) **Developing *Drosophila suzukii* management programs for sweet cherry in the western United States.** *Pest Management Science* 67:1386–1395.
- Bogwitz MR, Chung H, Magoc L, Rigby S, Wong W, O’Keefe M, McKenzie JA, Batterham P, Daborn PJ (2005) **Cyp12a4 confers lufenuron resistance in a natural population of *Drosophila melanogaster*.** *Proceedings of the National Academy of Sciences* 102:12807–12812.
- 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.
- Campos MR, Rodrigues ARS, Silva WM, Silva TBM, Silva VRF, Guedes RNC, Siqueira HAA (2014) **Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance.** *Plos ONE* 9:e103235.
- Croft BA (1990) **Developing a philosophy and program of pesticide resistance management.** In: *Pesticide Resistance in Arthropods*, Springer, US pp. 277–296.
- Daane KM, Wang XG, Biondi A, Miller B, Miller JC, Riedl H, Shearer PW, Guerrieri E, Giorgini M, Buffington M, van Achterberg K, Song Y, Kang T, Yi H, Jung C, Lee DW, Chung BK, Hoelmer KA, Walton VM (2016) **First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents.** *Journal of Pest Science* 89:823–835.
- Daborn P, Boundy S, Yen J, Pittendrigh B, French Constant R (2001) **DDT resistance in *Drosophila* correlates with Cyp6g1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid.** *Molecular Genetics and Genomics* 266:556–563.
- 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.
- Devonshire AL, Field LM, Foster SP, Moores GD, Williamson MS, Blackman RL (1998) **The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*.** *Philosophical Transactions of the Royal Society B* 353.
- Elzaki MEA, Miah MA, Wu M, Zhang H, Pu J, Jiang L, Han Z (2017) **Imidacloprid is degraded by CYP353D1v2, a cytochrome P450 overexpressed in a resistant strain of *Laodelphax striatellus*.** *Pest Management Science* 73:1358–1363.
- Emiljanowicz LM, Ryan GD, Langille A, Newman J (2014) **Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet.** *Journal of Economic Entomology* 107:1392–1398.

- Guedes RNC (2017) **Insecticide resistance, control failure likelihood and the First Law of Geography.** *Pest Management Science* 73:479–484.
- Guerrieri E, Giorgini M, Cascone P, Carpenito S, van Achterberg C (2016) **Species diversity in the parasitoid genus *Asobara* (Hymenoptera: Braconidae) from the native area of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae).** *Plos ONE* 11:e0147382.
- Haddi K, Berger M, Bielza P, Rapisarda C, Williamson MS, Moores G, Bass C (2017) **Mutation in the ace-1 gene of the tomato leaf miner (*Tuta absoluta*) associated with organophosphates resistance.** *Journal of Applied Entomology* p. 10.1111/jen.12386.
- Haddi K, Berger M, Bielza P, Cifuentes D, Field LM, Gorman K, Rapisarda C, Williamson MS, Bass C (2012) **Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*).** *Insect Biochemistry and Molecular Biology* 42:506–513.
- Haddi K, Tomé HVV, Du Y, Valbon WR, Nomura Y, Martins GF, Dong K, Oliveira EE (2017) **Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of *Aedes aegypti*: a potential challenge for mosquito control.** *Scientific Reports* 7:46549.
- Hamby KA, Kwok RS, Zalom FG, Chiu JC (2013) **Integrating circadian activity and gene expression profiles to predict chronotoxicity of *Drosophila suzukii* response to insecticides.** *Plos ONE* 8:e68472.
- Hampton E, Koski C, Barsoian O, Faubert H, Cowles RS, Alm SR (2014) **Use of early ripening cultivars to avoid infestation and mass trapping to manage *Drosophila suzukii* (Diptera: Drosophilidae) in *Vaccinium corymbosum* (Ericales: Ericaceae).** *Journal of Economic Entomology* 107:1849–1857.
- Haviland DR, Beers EH (2012) **Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries.** *Journal of Integrated Pest Management* 3:F1–F6.
- Haye T, Girod P, Cuthbertson AGS, Wang XG, Daane KM, Hoelmer KA, Baroffio C, Zhang JP, Desneux N (2016) **Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world.** *Journal of Pest Science* 89:643–651.
- IRAC (2011) **Susceptibility Test Method 026 - *Musca domestica*.** *Test Methods* <http://www.irc-online.org/methods/musca-domestica-adults/>:Last checked: 13 March 2016.
- Jang M, Kim J, Yoon KA, Lee SH, Park CG (2017) **Biological activity of Myrtaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae).** *Pest Management Science* 73:404–409.
- Kaplanoglu E, Chapman P, Scott IM, Donly C (2017) **Overexpression of a cytochrome P450 and a UDP-glycosyltransferase is associated with imidacloprid resistance in the Colorado potato beetle, *Leptinotarsa decemlineata*.** *Scientific Reports* 7:1762.
- Kenis M, Tonina L, Eschen R, van der Sluis B, Sancassani M, Mori N, Haye T, Helsen H (2016) **Non-crop plants used as hosts by *Drosophila suzukii* in Europe.** *Journal of Pest Science* 89:735–748.

- Liu Z, Williamson MS, Lansdell SJ, Han Z, Denholm I, Millar NS (2006) **A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides.** *Journal of Neurochemistry* 99:1273–1281.
- MAPA (2017) **AGROFIT - Sistema de Agrotóxicos Fitossanitários.** Ministério da Agricultura Pecuária e Abastecimento http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons:Last checked: 27 March 2017.
- Mishra R, Chiu JC, Hua G, Tawari NR, Adang MJ, Sial AA (2017) **High throughput sequencing reveals *Drosophila suzukii* responses to insecticides.** *Insect Science* n/a:10.1111/1744-7917.12498.
- Perry T, Heckel DG, McKenzie JA, Batterham P (2008) **Mutations in D α 1 or D β 2 nicotinic acetylcholine receptor subunits can confer resistance to neonicotinoids in *Drosophila melanogaster*.** *Insect Biochemistry and Molecular Biology* 38:520–528.
- Pittendrigh B, Reenan R, French Constant RH, Ganetzky B (1997) **Point mutations in the *Drosophila* sodium channel gene para associated with resistance to DDT and pyrethroid insecticides.** *Molecular and General Genetics* 256:602–610.
- Robertson JL, Savin N, Preisler HK, Russell RM (2007) **Binary quantal response with one explanatory variable.** In: *Bioassays with Arthropods, 2nd Edition.* CRC Press, Boca Raton, FL pp. 21–34.
- Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouil M, Bassi A (2015) **First report of *Tuta absoluta* resistance to diamide insecticides.** *Journal of Pest Science* 88:9–16.
- SAS Institute, (2013) **Statistical analysis system: Getting started with the SAS learning.** SAS Institute, Cary, NC p. 81.
- Silva WM, Berger M, Bass C, Balbino VQ, Amaral MHP, Campos MR, Siqueira HAA (2015) **Status of pyrethroid resistance and mechanisms in Brazilian populations of *Tuta absoluta*.** *Pesticide Biochemistry and Physiology* 122:8–14.
- Smirle MJ, Zurowski CL, Ayyanath MM, Scott IM, MacKenzie KE (2017) **Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura)(Diptera: Drosophilidae) populations from British Columbia, Canada.** *Pest Management Science* 73:130–137.
- Sparks TC, Nauen R (2015) **IRAC: Mode of action classification and insecticide resistance management.** *Pesticide Biochemistry and Physiology* 121:122–128.
- Tochen S, Dalton DT, Wiman N, Hamm C, Shearer PW, Walton VM (2014) **Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry.** *Environmental Entomology* 43:501–510.
- 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.
- Zhong D, Chang X, Zhou G, He Z, Fu F, Yan Z, Zhu G, Xu T, Bonizzoni M, Wang MH (2013) **Relationship between knockdown resistance, metabolic detoxification and organismal resistance to pyrethroids in *Anopheles sinensis*.** *Plos ONE* 8:e55475.

Chapter 4

Toxicity and egg-laying avoidance on *Drosophila suzukii* (Diptera: Drosophilidae) caused by an old alternative inorganic insecticide preparation

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Toxicity and egg-laying avoidance on *Drosophila suzukii* (Diptera: Drosophilidae) caused by an old alternative inorganic insecticide preparation

Abstract

The application of synthetic insecticides remains the most used tool for the management of spotted-wing *Drosophila*, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). However, the management of this pest in the organic production of soft-skinned fruits is a complex task due to the restricted number of registered products. Here, we assessed the toxicity of lime-sulfur and evaluated whether lime-sulfur-treated strawberry plants affected the oviposition and development of *D. suzukii*. The lime-sulfur exhibited adequate toxicity to *D. suzukii* ($LC_{50} = 26.6$ mL/L) without phytotoxicity to strawberry plants. When *D. suzukii* females were exposed to lime-sulfur-treated plants in no-choice bioassays, oviposition was significantly (*t*-test, $P < 0.05$) reduced compared with that on untreated plants. In free-choice bioassays, *D. suzukii* females laid significantly (paired *t*-test, $P < 0.05$) more eggs on untreated plants. Furthermore, in the free-choice bioassays, immature development was slower for adults that originated from eggs laid on lime-sulfur-treated plants than from those laid on untreated plants. Lime-sulfur showed adequate control and, therefore, has potential for use as a management tool against *D. suzukii* infestations in organic production systems. This old, alternative insecticide preparation caused not only adult fly mortality but also reduced the number of eggs laid on lime-sulfur-treated plants.

Keywords: spotted-wing *Drosophila*, lime-sulfur, alternative pest control, organic production systems.

4.1 Introduction

Successful organic cultivation of soft-skinned fruits (e.g., strawberries) requires effective and economical control of insect pests. However, because synthetic insecticides are not permitted in organic fruit production systems (Winter and Davis 2006) and because of the continued requirement for more environmentally benign pest control methods (coupled with regulatory actions to reduce the risks associated with these methods), the return to old inorganic insecticide preparations has recently gained popularity in agriculture, particularly in organic production (Williams and Cooper 2004; EPA 2005; Turra et al. 2014; Dahlawi and Siddiqui 2017).

The application of inorganic preparations rich in calcium polysulfides [under the general term “lime-sulfur” and easily prepared by combining water (H₂O) with elemental sulfur (S) and hydrated lime (CaO·H₂O) (Tartar 1914; Auld 1915)] has been recommended since the 19th century, representing one of the oldest effective pest (including insects) control methods (McCallan 1967; EPA 2005). Furthermore, because the ecological impacts of lime-sulfur preparations are apparently low and they are highly compatible with organic management practices (Williams and Cooper 2004; Russell 2005; Turra et al. 2014), their use continues to be commonly recommended in integrated pest management programs. Additionally, lime-sulfur preparations are one of most common recommendations to control fungi and mite infestations in organic production systems (Russell 2005; Penteado 2010; Venzon et al. 2013).

In the global production of strawberries, as observed for fields of other soft-skinned fruits (Bolda et al. 2010; Walsh et al. 2011; Steffan et al. 2013; Hamby et al. 2014), recent invasions of the spotted-wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), have elevated these flies to one of the most prevalent strawberry pests (Santos 2014; Asplen et al. 2015; Bernardi et al. 2015; Andreatza et al. 2016b). However, despite recent efforts aimed at developing other management strategies [e.g., potential parasitoids (Chabert et al. 2012; Poyet et al. 2013; Daane et al. 2016; Guerrieri et al. 2016), mass trapping (Hampton et al. 2014) and physical exclusion (netting) (Kawase et al. 2007)], the application of synthetic insecticides remains the most used tool for the management of *D. suzukii* (Bruck et al. 2011; Asplen et al. 2015; Andreatza et al. 2017). As a result, the management of organic production becomes an even more complex

task due to the restricted number of registered products.

Recent investigations evaluated the potential of using alternative insecticides (Gargani et al. 2013; Van Timmeren and Isaacs 2013), including sulfur-based preparations (Pérez-Guerrero and Molina 2016; Andreatza et al. 2017), to manage infestations of *D. suzukii*. However, although some sulfur-based commercial preparations did not provide adequate toxicity (Andreatza et al. 2017) against *D. suzukii* adults, the application of sulfur powder reduces the oviposition of *D. suzukii* under laboratory conditions (Pérez-Guerrero and Molina 2016). Thus, the present investigation was conducted aiming to evaluate the toxicity of inorganic lime-sulfur preparations to *D. suzukii* and to determine whether lime-sulfur-treated strawberry plants would affect the oviposition and development of *D. suzukii*.

4.2 Material and Methods

4.2.1 Insects and alternative insecticide preparations

The *D. suzukii* adult flies used in the bioassays were obtained from a stock colony reared under controlled conditions (i.e., temperature: 24 ± 2 °C; relative humidity: $55 \pm 10\%$; photophase: 12 h). The fly stock colony was initially established from approximately 800 individuals obtained from a colony maintained at Embrapa Clima Temperado (Pelotas, RS, Brazil). All developmental stages of the fly stock colony were reared following methods previously described elsewhere (Emiljanowicz et al. 2014; Andreatza et al. 2016a).

Three alternative insecticide preparations containing ingredients that are used to manage insect pests in organic systems (Liu and Stansly 2000; Venzon et al. 2013; EPA 2014; Marques-Francovig et al. 2014) were used in toxicological bioassays with *D. suzukii* adults. The lime-sulfur preparations were prepared according to the methodology described elsewhere (Guerra 1985; Penteado 2010; Venzon et al. 2006; Soto et al. 2010). Briefly, a stock solution with a final density of 32 ° Baumé was prepared from which further dilutions were performed before the applications. The other two preparations were prepared according to a handout of 'natural' recipes empirically used by organic farmers in southeast Brazil. A potassium permanganate (0.15%) + lime (1.0%) mixture was prepared from permanganate potassium powder (Ref.: 02690;

Neon Comercial Ltda, São Paulo - SP, Brazil), lime (bought at a local market) and distilled water at the proportions of 1:6.7:667, respectively. The third preparation (i.e., sulfur [0.15%] + salt [1.5%] + detergent [1.0%]) was a solution composed of sulfur powder (1.5 g), sodium chloride (NaCl, 15 g), and detergent (10 mL) mixed in one liter (1L) of distilled water.

4.2.2 Concentration–mortality bioassays

Lethal concentrations (LC) of the alternative insecticide preparations were estimated for *D. suzukii* using concentration-mortality bioassays. These toxicological bioassays were conducted using a contact/ingestion exposition protocol adapted from the IRAC Susceptibility Test Method No:026 (IRAC 2011). Briefly, a dental wick (Cremer S.A., Blumenau - SC, Brazil) was impregnated with 1.9 mL of the insecticide solution and placed inside a 200 mL glass vial. The vials were sealed at the top by a foam plug to prevent fly escape. After placement of the insecticide-impregnated dental wicks, groups of 25 mated flies were released into the glass vials for 24 h after which the mortality levels were assessed. A complete absence of movement after mechanical stimulation by a fine-tipped brush indicated a dead fly. For the bioassays with each alternative insecticide preparation, preliminary bioassays were conducted across a broad range (i.e., 1 to 1000 mL/L) to select the highest concentration with no mortality and the lowest concentration that did kill all *D. suzukii*. For each insecticide concentration, four replicates (i.e., each replicate a vial containing 25 flies and the insecticide-impregnated dental wick) were used to determine mortality.

4.2.3 Lime-sulfur effect on egg-laying avoidance (with and without choice) under semi-field conditions

To assess the activity of the lime-sulfur on female oviposition behavior, flies were provided with choice and no-choice oviposition bioassays under semi-field conditions. Strawberry plants were cultivated in plastic pots (4 L) in the greenhouse to avoid previous *D. suzukii* infestation. When each had three ripe fruits, the plants were sprayed with the lime-sulfur preparation at 30 mL/L (approximately the LC₅₀ estimated for *D. suzukii* in the present investigation), a commonly recommended concentration for mite pest control (Hassan et al. 1994; Silva et al. 2009; Penteadó 2010).

The untreated (i.e., control) plants were sprayed with distilled and deionized water. To spray the plants, a hand sprayer (0.5 L) was pressed 15 times around the plant, which resulted in a deposition of approximately 10 mL of solution per plant. The pots with lime-sulfur-treated and untreated plants were then transferred to the field and partially buried, placing the plants close to the ground level.

For the no-choice oviposition bioassay, groups of four untreated or four lime-sulfur-treated plants were transferred to the field, and a cage of plastic pipe (2.5 cm in diameter) covered with organza (1.2 × 1.2 × 0.8 m; Figure 4.1) was immediately placed over the plants. The four plants were transplanted in different corners of the cage. On the top of each cage, a zipper allowed access to the inside through which 30 mated *D. suzukii* females (3-4 days old) were released. The flies were released 1 h after the plants were caged. In the free-choice oviposition bioassay (Figure 4.1), two plants were lime-sulfur-treated and two were untreated. Of note, during the semi-field experiments (August 2016), no rain fell and daylight length remained between 11.2 and 11.4 h. The mean temperature ranged from 19.7 to 20.9 °C, and the relative humidity range was 69-73%. The cage locations received approximately 70% of the daylight length under direct sunlight, with cages in an open, well ventilated environment. In both oviposition bioassay types, 24 h after the release of flies, the strawberry fruits were harvested, and the oviposited eggs were counted using a stereoscopic microscope. In each bioassay type, five replicates (i.e., a group of four plants) were used.

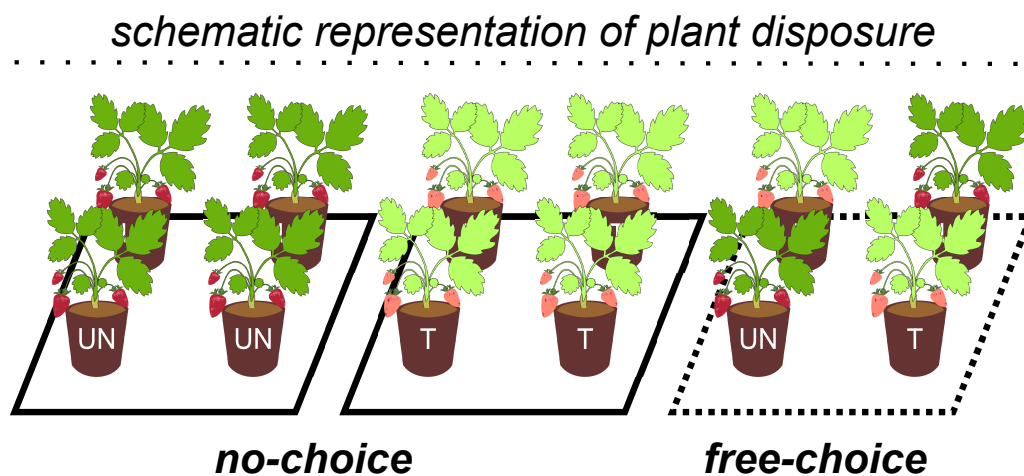


Figure 4.1: Schematic representation of the experimental setup for the lime-sulfur egg laying avoidance (with and without choice) bioassays.

4.2.4 Pre-imaginal development and emergence bioassays

After the egg counts, the strawberry fruits were kept in plastic containers (0.5 L) on a 1 cm layer of vermiculite (Ref.: 23071; Caldesul Co., Porto Alegre - RS, Brazil) for egg hatch and immature development. The plastic containers were closed at the top with a sleeve-like voile fabric. From the 8th to 16th day after oviposition, all emerged adults were collected and sexed daily.

4.2.5 Statistical analyses

Concentration–mortality curves were estimated with probit analyses using the PROBIT procedure in the SAS statistical software package (SAS Institute, 2013). The number of eggs laid, adult emergence, the sex ratio, and egg-to-adult viability from each cage in the no-choice oviposition bioassay were analyzed by a Student's *t*-test or a Mann-Whitney Rank Sum test when assumptions of normality and homoscedasticity were not satisfied. All comparisons were performed using SigmaPlot 12.5 (Systat Software, San Jose, CA, USA). For the free-choice oviposition bioassay, the same parameters were analyzed by a paired Student's *t*-test on Sigma Plot 12.5. Additionally, the daily number of emerged adults and the daily emergence percentage in each treatment were submitted to nonlinear regression analysis using the curve fitting procedure of Sigma Plot 12.5. The regression analyses were conducted to detect trends in the pre-imaginal development period that resulted from the lime-sulfur treatment or the choice of female oviposition. The regression models for each treatment were considered significantly different when the confidence limits of their parameters did not overlap.

4.3 Results

4.3.1 Concentration–mortality bioassays

Of the three tested alternative insecticide preparations, only the lime-sulfur solution increased mortality in response to increasing concentrations (Figure 4.2). As shown in Table 4.1, the lime-sulfur LC₅₀ value was 26.6 (24.3-29.6) mL/L, which is approximately the recommended concentration (i.e., 30 mL/L) for controlling mite pests in

Neotropical organic production systems. However, with the highest concentration (i.e., 1000 mg/mL) of both potassium permanganate + lime and sulfur + salt + detergent preparations, mortality did not exceed $11.3 \pm 2.3\%$ and $6.5 \pm 3.3\%$ of the tested flies, respectively.

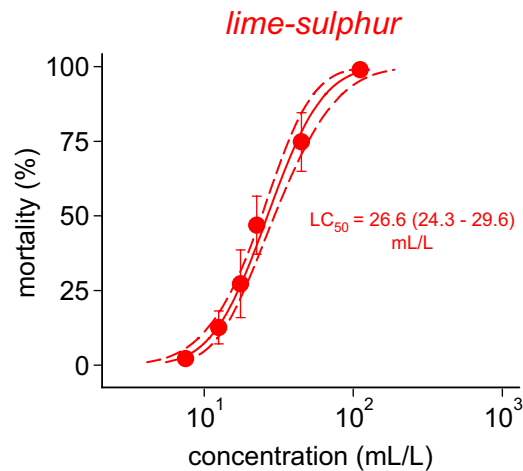


Figure 4.2: Toxicity of lime-sulfur to adults of *Drosophila suzukii*. The lines denote the lethal concentration (LC) values estimated based on concentration-mortality bioassays using probit analyses. Symbols show the averaged mortality recorded for each insecticide concentration. The vertical bars represent the standard error of the average (SE).

Table 4.1: Toxicity of lime-sulfur to *Drosophila suzukii* adults ($n = 502$, $\chi^2 = 2.3$, $df = 4$; $P = 0.51$). Lethal concentrations (LC) values were estimated based on concentration-mortality bioassays using probit analyses. C.I. denotes confidence interval. All concentrations are expressed in mL of lime-sulfur preparation/L of water.

Slope \pm SE	LC ₂₀ (95% C.I. ^a)	LC ₅₀ (95% C.I.)	LC ₈₀ (95% C.I.)
3.4 ± 0.30	15.1 (13.4 - 16.6)	26.6 (24.3 - 29.6)	47.0 (40.6 - 57.1)

4.3.2 Semi-field oviposition bioassays (with and without choice)

In the no-choice bioassay, the groups of 30 females released into each cage with lime-sulfur-treated plants laid 18.5 ± 4.4 eggs/plant, which was significantly fewer eggs ($t = 3.04$, $df = 8$, $P = 0.016$) than the 35.3 ± 3.4 eggs laid per each untreated plant (Figure 4.3A). Similarly, in the choice bioassay, the groups of 30 females released in each cage laid 20.0 ± 4.8 eggs on each lime-sulfur-treated plant, which was $48.6 \pm 9\%$ fewer eggs ($t = 4.44$, $df = 4$, $P = 0.011$) than the 38.0 ± 3.7 eggs laid on each untreated plant (Figure

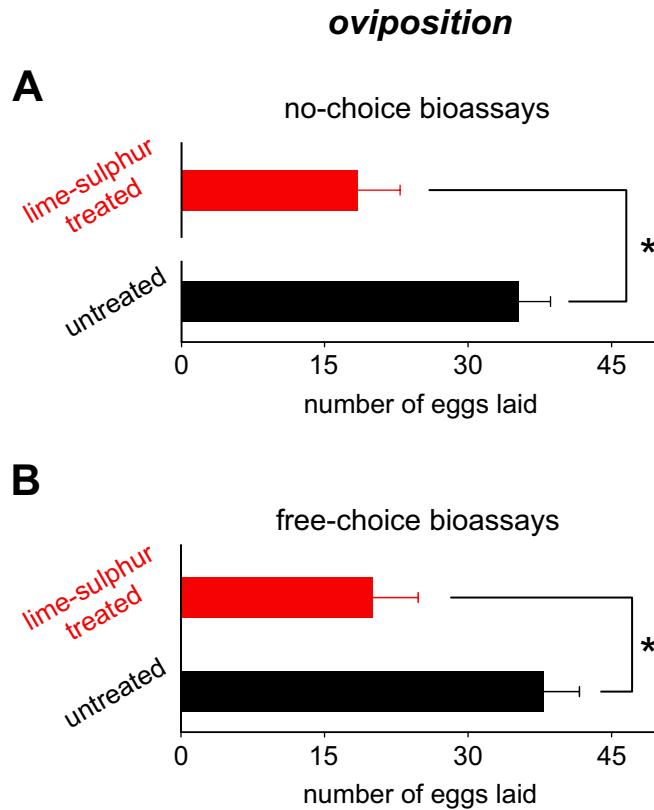


Figure 4.3: Total number of eggs laid per plant by *Drosophila suzukii* females on lime-sulfur-treated or untreated strawberry plants under semi-field conditions. (A) Sexually mature *D. suzukii* females were confined in cages containing four lime-sulfur-treated or four untreated strawberry plants in a no-choice bioassay. (B) The female flies were subjected to a choice bioassay under semi-field conditions and were confined in cages containing four strawberry plants, with two lime-sulfur-treated and two untreated plants. Asterisks indicate significant differences between treatments according to Student's *t*-test (A) or paired Student's *t*-test (B) ($P < 0.05$).

4.3B).

4.3.3 Pre-imaginal development and emergence bioassays

In the no-choice oviposition bioassay, the egg-to-adult viability (Mann-Whitney rank sum test; $P = 0.31$) and the offspring female sex ratios (Mann-Whitney rank sum test; $P = 0.69$) were not significantly different between the eggs laid on lime-sulfur-treated plants and those on untreated plants. Additionally, no significant differences (*Egg-to-adult viability*: Mann-Whitney rank sum test, $P = 0.55$; *Offspring sex ratio*: paired *t*-test, $P = 0.69$) were recorded for these parameters for the eggs laid on both plant types in the choice oviposition bioassay. The overall egg-to-adult viability was $94.4 \pm 2.3\%$, and the female sex ratio was $49.6 \pm 2.0\%$.

The cumulative number of flies developed from eggs laid on lime-sulfur-treated

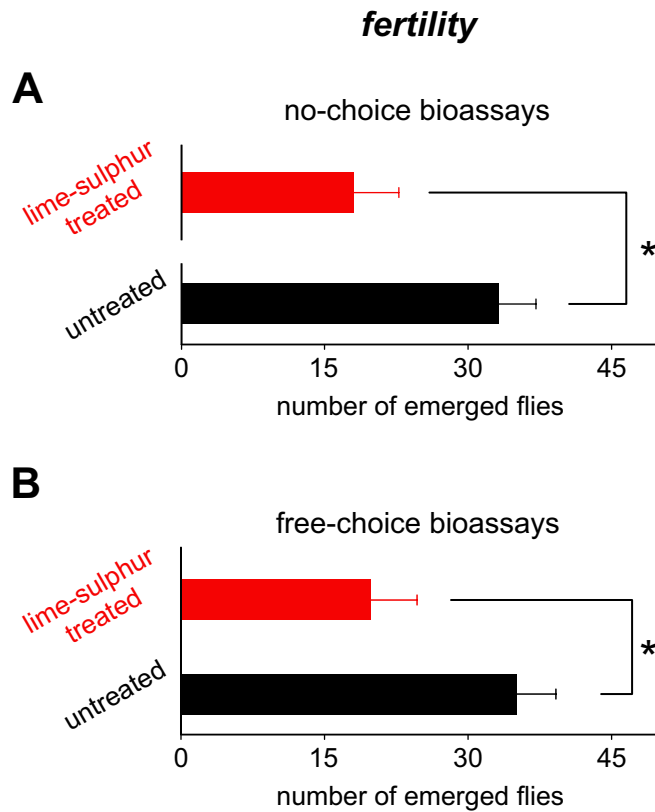


Figure 4.4: Number of emerged *Drosophila suzukii* adults originated from eggs laid on lime-sulfur-treated or untreated strawberry plants. (A) Adults emerged from eggs laid on lime-sulfur-treated or untreated strawberry plants in a no-choice bioassay. (B) Adults emerged from eggs laid on lime-sulfur-treated or untreated strawberry plants in a free-choice bioassay. Asterisks indicate significant differences between treatments according to Student's *t*-test (A) or paired Student's *t*-test (B) ($P < 0.05$).

plants was significantly ($t = 2.48$, $df = 8$, $P = 0.038$) lower than the number that developed from eggs laid on untreated plants in the no-choice oviposition bioassay (Figure 4.4A). As shown in Figure 4.4B, in the choice oviposition bioassay, the cumulative number of flies developed from eggs laid on lime-sulfur-treated plants was also significantly ($t = 5.48$, $df = 4$, $P = 0.005$) fewer than that developed from eggs laid on untreated plants.

Although the peak occurrence for daily emergence both in numbers and in percentages was not significantly different between lime-sulfur-treated and untreated plants in the no-choice oviposition bioassay (Table 4.2, Figure 4.5A,B), these peak occurrences were delayed for eggs laid on lime-sulfur-treated plants in the choice oviposition bioassay (Table 4.2, Figure 4.5C,D).

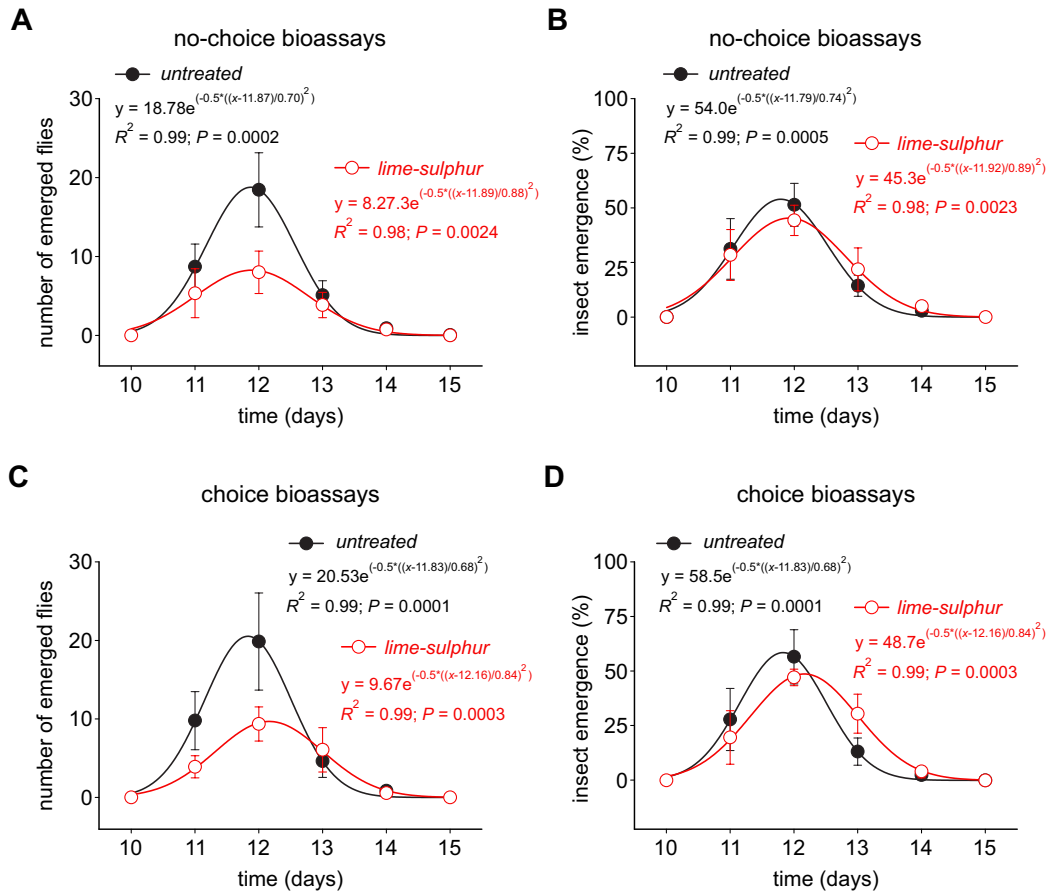


Figure 4.5: The daily emergency of *D. sukuzii* adults in number (A, C) and in percentage of emerged flies (B, D) that were developed from eggs laid on lime-sulfur-treated or untreated plants in oviposition bioassays with and without free-choice. Symbols represent means of the observed data and lines represent the fits to the data.

Table 4.2: Summary of the model parameters obtained from the Gaussian peak nonlinear regression curves shown in Fig. 5.

Parameters ^a	Treatment	No-choice bioassay			Choice bioassay		
		value (95% C.L.) ^b	<i>t</i> -value	<i>P</i>	value (95% C.L.) ^b	<i>t</i> -value	<i>P</i>
daily emergence in number of emerged flies							
<i>a</i>	untreated	18.78 (17.10 - 20.46)a	35.5	<0.0001	20.53 (18.76 - 22.30)a	36.9	<0.0001
	lime-sulfur treated	8.27 (6.52 - 10.01)b	15.1	0.0006	9.67 (8.64 - 10.71)b	29.6	<0.0001
<i>b</i>	untreated	0.70 (0.63 - 0.77)a	32.4	<0.0001	0.68 (0.61 - 0.74)a	32.9	<0.0001
	lime-sulfur treated	0.88 (0.67 - 1.09)a	13.3	0.0009	0.84 (0.74 - 0.94)a	26.1	0.0001
<i>X</i> ₀	untreated	11.87 (11.79 - 11.96)a	456.3	<0.0001	11.83 (11.75 - 11.91)a	492.7	<0.0001
	lime-sulfur treated	11.89 (11.67 - 12.11)a	172.5	<0.0001	12.16 (12.06 - 12.27)b	363.2	<0.0001
daily emergence in percentage of emerged flies							
<i>a</i>	untreated	53.97 (46.69 - 61.24)a	23.6	0.0002	58.49 (53.46 - 63.52)a	37.0	<0.0001
	lime-sulfur treated	45.34 (35.99 - 54.70)a	15.4	0.0006	48.71 (43.48 - 53.94)a	29.6	<0.0001
<i>b</i>	untreated	0.74 (0.62 - 0.85)a	20.7	0.0002	0.68 (0.61 - 0.74)a	32.9	<0.0001
	lime-sulfur treated	0.89 (0.68 - 1.10)a	13.6	0.0009	0.84 (0.74 - 0.94)a	26.1	0.0001
<i>X</i> ₀	untreated	11.79 (11.67 - 11.90)a	313.3	<0.0001	11.83 (11.75 - 11.91)a	492.7	<0.0001
	lime-sulfur treated	11.92 (11.71 - 12.14)a	175.6	<0.0001	12.16 (12.06 - 12.27)b	363.2	<0.0001

^a Coefficients from a three-parameters Gaussian peak nonlinear regression model: $Y = a * \exp(-0.5 * ((X-X_0)/b)^2)$; *a* = maximum peak value at Y-axis (number of emerged flies); *b* = rate of change; *X*₀ = location of the peak on the X-axis (time). ^b Different letters on a column within each parameter indicate significant differences due to non-overlap of the 95% confidence limits (C.L.).

4.4 Discussion

The application of sulfur-based insecticide preparations is an old tool commonly used to manage fungi, mite and insect infestations in organic production systems (Hassan et al. 1994; Russell 2005; Afonso et al. 2007; Venzon et al. 2013; Marques-Francovig et al. 2014). Here, we demonstrated the lime-sulfur insecticide preparation was toxic to *D. suzukii* adults and also caused egg-laying avoidance in *D. suzukii* females without resulting in phytotoxic effects on strawberry plants.

The natural degradation of lime-sulfur preparations is well known to result in both hydrogen sulfides (H₂S) and elemental sulfur, which are toxicologically active against pests (Abbott 1945). Whereas hydrogen sulfides inhibit cytochrome oxidase, elemental sulfur oxidizes cytochrome b to cytochrome c with a by-product release of H₂S (Smith et al. 1977; Dorman et al. 2002; Dahlawi and Siddiqui 2017). Although not addressed in detail in this study, a reasonable assumption is that hydrogen sulfides exerted the primary toxic actions against *D. suzukii*, because in previous investigations (Andreazza et al. 2017), other sulfur-based preparations did not provide adequate control of *D. suzukii*. Furthermore, the other sulfur-based preparation (i.e., sulfur + NaCl + detergent) tested in the present investigation also resulted in very low mortality to *D. suzukii*, even when applied pure (i.e., without further dilution). Notably, although the potassium permanganate + lime preparation is recognized as an alternative preparation with potent fungicide, bactericide and molluscicide activities (EPA 2014), the application of pure (i.e., without further dilution) preparation resulted in very low mortality to *D. suzukii*. Thus, the potential use of these two preparations in the management of *D. suzukii* infestations in the organic production of soft-skinned fruits is completely excluded.

Although pesticide products with a limited spectrum of activity may contribute to the safety of the environment and non-target organisms, such traits increase the complexity for alternative pesticides to enter niche markets and for the development and commercialization of these products (Axel et al. 2012; Glare et al. 2012; Isman and Grieneisen 2014). Therefore, to encourage the widespread use of alternative pesticides, products with activity against multiple pests are required. The application of the lime-sulfur preparation represents a good example of an alternative product ca-

pable of controlling multiple insect pests in the organic production of strawberries. In strawberry production systems worldwide, phytophagous mites [particularly the two-spotted spider mite, *Tetranychus urticae* (Koch) (Solomon et al. 2001; Klingen and Westrum 2007; Moraes and Flechtmann 2008)], and more recently, *D. suzukii* are considered the most prevalent pests (Santos 2014; Asplen et al. 2015; Bernardi et al. 2015; 2017), and both pest systems can be adequately controlled by lime-sulfur preparations. For example, the lime-sulfur LC₈₀ for *D. suzukii* is in the concentration range known to provide adequate control of *T. urticae* (Marques-Francovig et al. 2014).

Similar to the effect recorded for sulfur powder applications (Pérez-Guerrero and Molina 2016), the application of the lime-sulfur preparation on strawberry plants resulted in egg-laying avoidance in *D. suzukii* females in the no-choice oviposition bioassay. Because drosophilids have relatively short generation times and high reproductive outputs (e.g., with several overlapping generations during each crop season) (Dreves et al. 2009; Emiljanowicz et al. 2014; Tochen et al. 2014), the reductions in the number of eggs laid will not fully prevent damage to strawberry fruits; however, such a reduction certainly has negative effects on population growth of the fly, and therefore, the lime-sulfur preparation could maintain the size of the fly population below the economic threshold. Furthermore, by reducing egg-laying, the application of lime-sulfur preparations can be integrated with other “push-pull” strategies (e.g., the use of attractive baits or alternative plant hosts) (Miller and Cowles 1990), which may favor the management of *D. suzukii* by increasing fly dispersion to other areas than the strawberry fields. Reinforcing the potential of the lime-sulfur preparation as a “push-pull” strategy component, we also demonstrated that *D. suzukii* females laid significantly more eggs on untreated plants in the free-choice oviposition bioassay.

Site selection for egg-laying is a central behavioral decision for insects that is strongly affected by genetic and environmental factors, with the decision strongly affecting lifetime reproductive fitness by favoring (or not) offspring survival (Yang et al. 2008; Joseph et al. 2009; Abed-Vieillard et al. 2013; Battesti et al. 2015; Bezzar-Bendjazia et al. 2016). In this study, we recorded not only a reduction in the number of eggs laid but also a significant delay in the emergence peak for the individuals from eggs laid on lime-sulfur-treated plants. These responses to lime-sulfur treatment might be a consequence of the decisions of *D. suzukii* females, as proposed in the theory of optimal

oviposition behavior (Jaenike 1978; Schoonhoven et al. 2005), and reflect the abilities of drosophilid females to actively probe the environment and evaluate site quality before depositing each egg (Yang et al. 2008; Joseph et al. 2009; Abed-Vieillard et al. 2013; Battesti et al. 2015; Bezzar-Bendjazia et al. 2016).

Thus, in the present investigation, the potential of using an old, alternative insecticide preparation (i.e., lime-sulfur) was demonstrated as a *D. suzukii* management tool in organic production of soft-skinned fruits (e.g., strawberries). Further investigations should evaluate the contributions of environmental (e.g., social cues coming from conspecifics or presence of aversive tastants) and genetic factors (e.g., neural basis that governs simple decision-making processes) to the preference for oviposition sites, which will contribute to a better understanding of the sublethal effects induced by lime-sulfur on *D. suzukii* females.

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References

- Abbott CE (1945) **The toxic gases of lime-sulfur.** *Journal of Economic Entomology* 38:618–620.
- Abed-Vieillard D, Cortot J, Everaerts C, Ferveur JF (2013) **Choice alters *Drosophila* oviposition site preference on menthol.** *Biology Open* p. BIO20136973.
- Afonso A, Faria J, Botton M, Zanardi O (2007) **Avaliação da calda sulfocálcica e do óleo mineral no controle da cochonilha-parda *Parthenolecanium persicae* (Hemiptera: Coccidae) na cultura da videira.** *Arquivos do Instituto Biológico* 74:167–169.
- Andreazza F, Bernardi D, Baronio CA, Pasinato J, Nava DE, Botton M (2017) **Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae).** *Pest Management Science* 73:146–152.
- Andreazza F, Bernardi D, Marangon RB, Scheunemann T, Botton M, Nava DE (2016a)

- Técnica de criação de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) em dieta artificial.** *Boletim de Pesquisa e Desenvolvimento/Embrapa Clima Temperado* 240:23p.
- Andreazza F, Haddi K, Oliveira EE, Ferreira JAM (2016b) ***Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil.** *Florida Entomologist* 99:796–798.
- Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Kárpáti Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Véték G, Vogt H, Walton VM, Yu Y, Zappalà L, Desneux N (2015) **Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities.** *Journal of Pest Science* 88:469–494.
- Auld SJM (1915) **LVII.-The reaction between calcium hydroxide and sulphur in aqueous solution.** *Journal of the Chemical Society, Transactions* 107:480–495.
- Axel C, Zannini E, Coffey A, Guo J, Waters DM, Arendt EK (2012) **Ecofriendly control of potato late blight causative agent and the potential role of lactic acid bacteria: a review.** *Applied Microbiology and Biotechnology* 96:37–48.
- Battesti M, Moreno C, Joly D, Mery F (2015) **Biased social transmission in *Drosophila* oviposition choice.** *Behavioral Ecology and Sociobiology* 69:83–87.
- Bernardi D, Andreazza F, Botton M, Baronio CA, Nava DE (2017) **Susceptibility and interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in damaging strawberry.** *Neotropical Entomology* 46:1–7.
- Bernardi D, Botton M, Nava DE, Zawadneak MAC (2015) *Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro*, Vol. 1 Embrapa, Brasília - DF.
- Bezzar-Bendjazia R, Kilani-Morakchi S, Aribi N (2016) **Larval exposure to azadirachtin affects fitness and oviposition site preference of *Drosophila melanogaster*.** *Pesticide Biochemistry and Physiology* 133:85–90.
- Bolda MP, Goodhue RE, Zalom FG (2010) **Spotted wing drosophila: potential economic impact of a newly established pest.** *Agricultural and Resource Economics Update* 13:5–8.
- 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.
- Chabert S, Allemand R, Poyet M, Eslin P, Gibert P (2012) **Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, *Drosophila suzukii*.** *Advances in Parasitology* 63:40–47.
- Daane KM, Wang XG, Biondi A, Miller B, Miller JC, Riedl H, Shearer PW, Guerrieri E, Giorgini M, Buffington M, van Achterberg K, Song Y, Kang T, Yi H, Jung C, Lee DW, Chung BK, Hoelmer KA, Walton VM (2016) **First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents.** *Journal of Pest Science* 89:823–835.

- Dahlawi SM, Siddiqui S (2017) **Calcium polysulphide, its applications and emerging risk of environmental pollution-a review article.** *Environmental Science and Pollution Research* pp. 1–11.
- Dorman DC, Moulin FJM, McManus BE, Mahle KC, James RA, Struve MF (2002) **Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium.** *Toxicological Sciences* 65:18–25.
- Dreves AJ, Walton VM, Fisher G (2009) **A new pest attacking healthy ripening fruit in Oregon, Spotted wing *Drosophila*: *Drosophila suzukii* (Matsumura)** Technical report EM 8991, Oregon State University.
- Emiljanowicz LM, Ryan GD, Langille A, Newman J (2014) **Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet.** *Journal of Economic Entomology* 107:1392–1398.
- EPA (2005) **Office of Prevention, Pesticides and Other Toxic Substances. Reregistration eligibility decision for inorganic polysulfides.** list d—case no. 4054 .
- EPA (2014) **OPP Pesticide Ecotoxicity Database.** *Regional IPM Centers* <http://www.ipmcenters.org/Ecotox/DataAccess.cfm>.
- Gargani E, Tarchi F, Frosinini R, Mazza G, Simoni S (2013) **Notes on *Drosophila suzukii* Matsumura (Diptera: Drosophilidae): field survey in Tuscany and laboratory evaluation of organic products.** *Redia* 96:85–90.
- 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.
- Guerra MdS (1985) **Receituário caseiro: alternativas para o controle de pragas e doenças de plantas cultivadas e de seus produtos.** *Embrater* p. 166.
- Guerrieri E, Giorgini M, Cascone P, Carpenito S, van Achterberg C (2016) **Species diversity in the parasitoid genus *Asobara* (Hymenoptera: Braconidae) from the native area of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae).** *Plos ONE* 11:e0147382.
- Hamby KA, Bolda MP, Sheehan ME, Zalom FG (2014) **Seasonal monitoring for *Drosophila suzukii* (Diptera: Drosophilidae) in California commercial raspberries.** *Environmental Entomology* 43:1008–1018.
- Hampton E, Koski C, Barsoian O, Faubert H, Cowles RS, Alm SR (2014) **Use of early ripening cultivars to avoid infestation and mass trapping to manage *Drosophila suzukii* (Diptera: Drosophilidae) in *Vaccinium corymbosum* (Ericales: Ericaceae).** *Journal of Economic Entomology* 107:1849–1857.
- Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Calis JNM, Coremans-Pelseneer J, Duso C, Grove A, Heimbach U, Helyer N, Hokkanen H, Lewis GB, Mansour F, Moreth L, Polgar L, Samsøe-Petersen L, Sauphanor B, Stäubli A, Sterk G, Vainio A, van de Veire M, Viggiani G, Vogt H (1994) **Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group-pesticides and beneficial organisms.** *Entomophaga* 39:107–119.

- IRAC (2011) **Susceptibility Test Method 026 - *Musca domestica*.** *Test Methods* <http://www.irc-online.org/methods/musca-domestica-adults/>:Last checked: 13 March 2016.
- Isman MB, Grieneisen ML (2014) **Botanical insecticide research: many publications, limited useful data.** *Trends in Plant Science* 19:140–145.
- Jaenike J (1978) **On optimal oviposition behavior in phytophagous insects.** *Theoretical Population Biology* 14:350–356.
- Joseph RM, Devineni AV, King IF, Heberlein U (2009) **Oviposition preference for and positional avoidance of acetic acid provide a model for competing behavioral drives in *Drosophila*.** *Proceedings of the National Academy of Sciences* 106:11352–11357.
- Kawase S, Uchino K, Takahashi K (2007) **Control of cherry drosophila, *Drosophila suzukii*, injurious to blueberry.** *Plant Protection* 61:205–209.
- Klingen I, Westrum K (2007) **The effect of pesticides used in strawberries on the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) and its fungal natural enemy *Neozygites floridana* (Zygomycetes: Entomophthorales).** *Biological Control* 43:222–230.
- Liu TX, Stansly PA (2000) **Insecticidal activity of surfactants and oils against silver-leaf whitefly (*Bemisia argentifolii*) nymphs (Homoptera: Aleyrodidae) on collards and tomato.** *Pest Management Science* 56:861–866.
- Marques-Francovig CR, Mikami AY, Dutra V, Carvalho MG, Picareli B, Ventura MU (2014) **Fertilização orgânica e inseticidas botânicos para controle do ácaro rajado em morango.** *Ciência Rural* 44:7.
- McCallan SEA (1967) **History of fungicides.** In: *Fungicides: an advanced treatise*, Academic Press, NY pp. 1–37.
- Miller JR, Cowles RS (1990) **Stimulo-deterrent diversion: a concept and its possible application to onion maggot control.** *Journal of Chemical Ecology* 16:3197–3212.
- Moraes GJ, Flechtmann CHW (2008) **Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil.** *Holos* p. 288.
- Penteado SR (2010) **Controle alternativo de pragas e doenças com as caldas bordalesa, sulfocálcica e Viçosa.** *Via Orgânica* p. 152.
- Pérez-Guerrero S, Molina J (2016) **Laboratory approach to the use of sulphur and kaolin as preventive control against *Drosophila suzukii*.** *Spanish Journal of Agricultural Research* 14:10–01.
- Poyet M, Havard S, Prevost G, Chabrierie O, Doury G, Gibert P, Eslin P (2013) **Resistance of *Drosophila suzukii* to the larval parasitoids *Leptopilina heterotoma* and *Asobara japonica* is related to haemocyte load.** *Physiological Entomology* 38:45–53.
- Russell P (2005) **A century of fungicide evolution.** *The Journal of Agricultural Science* 143:11–25.
- Santos RSS (2014) **Ocorrência de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morango no Brasil.** *Comunicado Técnico/ Embrapa Uva e Vinho* 159:4.

- SAS Institute, (2013) **Statistical analysis system: Getting started with the SAS learning.** SAS Institute, Carry, NC p. 81.
- Schoonhoven LM, Van Loon JJ, Dicke M (2005) **Insect-plant biology.** Oxford University Press on Demand p. 440.
- Silva MZd, Oliveira CALd, Sato ME (2009) **Seletividade de produtos fitossanitários sobre o ácaro predador *Agistemus brasiliensis* Matioli, Ueckermann & Oliveira (Acari: Stigmaeidae).** *Revista Brasileira de Fruticultura* 31:388–396.
- Smith L, Kruszyna H, Smith RP (1977) **The effect of methemoglobin on the inhibition of cytochrome c oxidase by cyanide, sulfide or azide.** *Biochemical Pharmacology* 26:2247–2250.
- Solomon M, Jay C, Innocenzi P, Fitzgerald J, Crook D, Crook A, Easterbrook M, Cross J (2001) **Review: natural enemies and biocontrol of pests of strawberry in northern and central Europe.** *Biocontrol Science and Technology* 11:165–216.
- Soto A, Venzon M, Oliveira RM, Oliveira HG, Pallini A (2010) **Alternative control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on tomato plants grown in greenhouses.** *Neotropical Entomology* 39:638–644.
- Steffan SA, Lee JC, Singleton ME, Vilaire A, Walsh DB, Lavigne LS, Patten K (2013) **Susceptibility of cranberries to *Drosophila suzukii* (Diptera: Drosophilidae).** *Journal of Economic Entomology* 106:2424–2427.
- Tartar HV (1914) **The reaction between sulphur and calcium hydroxide in aqueous solution.** *Journal of American Chemistry Society* 36:495–498.
- Tochen S, Dalton DT, Wiman N, Hamm C, Shearer PW, Walton VM (2014) **Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry.** *Environmental Entomology* 43:501–510.
- Turra C, Fernandes EADN, Bacchi MA, Kato LS (2014) **Chemical composition of agricultural supplies used in Brazilian organic fruticulture.** *Biological Agriculture & Horticulture* 30:1–7.
- 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.
- Venzon M, Oliveira RM, Perez AL, Rodríguez-Cruz FA, Martins Filho S (2013) **Lime sulfur toxicity to broad mite, to its host plants and to natural enemies.** *Pest Management Science* 69:738–743.
- Venzon M, Rosado MdC, Pinto CMF, Duarte VdS, Euzébio DE, Pallini A (2006) **Potencial de defensivos alternativos para o controle do ácaro-branco em pimenta “Malagueta”.** *Horticultura Brasileira* 24:224–227.
- Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O’Neal SD, Zalom FG (2011) ***Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential.** *Journal of Integrated Pest Management* 2:G1–G7.
- Williams J, Cooper R (2004) **The oldest fungicide and newest phytoalexin—a reappraisal of the fungitoxicity of elemental sulphur.** *Plant Pathology* 53:263–279.

Winter CK, Davis SF (2006) **Organic Foods**. *Journal of Food Science* 71:R117–R124.

Yang Ch, Belawat P, Hafen E, Jan LY, Jan YN (2008) **Drosophila egg-laying site selection as a system to study simple decision-making processes**. *Science* 319:1679–1683.

General conclusions

Drosophila suzukii is already present in both, the Zona da Mata Mineira and Capixaba Highlands, in the states of Minas Gerais and Espírito Santo, Brazil, respectively.

Zaprionus indianus can occur in high numbers in association with *D. suzukii* in blackberries prior harvest.

The *Drosophila suzukii* population collected from Juiz de Fora was more resistant to imidacloprid than both the laboratory and Ervália populations, suggesting the need of studies and management strategies to avoid selection of highly resistant populations and future control failures.

There were no consistent insecticide susceptibilities differences among the population from Juiz de Fora, Ervália and laboratory to the insecticide permethrin.

Alternatively to the use of synthetic insecticide, lime-sulfur showed to be toxic to *D. suzukii* adults, and sprays in strawberry plants did reduced both the oviposition and number of descendants of *D. suzukii*, without exhibiting visually phytotoxicity. However, more testing evaluating the lime-sulfur phytotoxicity on strawberry plants and other *D. suzukii* hosts, not only visually, is necessary prior any field recommendation.

Both the alternative preparations using potassium permanganate + lime and sulfur + salt + detergent and the insecticides azadirachtin and indoxacard did not exhibit toxicity to *D. suzukii* in the laboratory bioassays, and therefore were considered ineffective to manage this pest species.