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&

UNIVERSITY OF GRONINGEN – UG
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GRONINGEN INSTITUTE FOR EVOLUTIONARY LIFE SCIENCES – GELIFES

DAHISE BRILINGER

INTEGRATED MANAGEMENT OF *Drosophila suzukii:* MONITORING AND MANAGEMENT STRATEGIES

LAGES, BR
GRONINGEN, NL
2024

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Presented to the Graduate Program in Crop Production at the Santa Catarina State University, as a partial requirement for obtaining the title of Doctor of Science in Crop Production.

and

To obtain the degree of PhD at the University of Groningen on the authority of the Rector Magnificus Prof. J. Scherpen and in accordance with the decision by the College of Deans.

Supervisors: Mari Inês Carissimi Boff (UDESC) and Bregje Wertheim (UG)

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Groningen, 17 May 2024

To my lovely family

Dalvani, Delei, Dehiert & Denilson

To my sweet nephews

Frederico Arnoldo & João Franciso

To my adorable cats

Lasanha and Tom

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“A possibilidade de realizarmos um sonho é o que torna a vida interessante.”

“It is the possibility of having a dream come true that makes life interesting.”

Paulo Coelho

ABSTRACT

BRILINGER, D. **Integrated management of *Drosophila suzukii*: monitoring and management strategies**. 2024. 166 f. Thesis (Doctorate in Crop Production) - Santa Catarina State University, Agroveterinary Sciences Centre, Graduate College in Crop Production, Lages. 2024.

The invasive fruit fly, *Drosophila suzukii*, has become a global threat to small fruit production due to its global expansion and biological characteristics. Its ability to produce seasonal morphotypes allows it to survive in various climates, leading to multiple generations per year. Females prefer soft-skinned fruits for oviposition, causing economic losses for growers as larvae feed on fruit pulp and create openings for pathogenic microorganisms. In regions like the Netherlands and Brazil, significant fruit losses have been recorded, affecting crops like small berries and grapes. This research explored different approaches to integrated pest management and contributes to the development of a management program for *D. suzukii*, focusing primarily on vineyards in São Joaquim, Santa Catarina, Brazil. Fieldwork monitoring revealed higher pest densities in forest borders compared to vineyards, with migration into vineyards during grape ripening. Climate variables, particularly relative humidity, significantly influenced pest captures, varying by microclimate in each vineyard. Laboratory evaluations highlighted grape cultivar-specific susceptibility to *D. suzukii*, emphasizing the importance of skin integrity and oviposition choice. Physical and chemical grape characteristics, such as skin resistance and pH, impact pest oviposition behaviors. Botanical compounds such as matrine and oxymatrine showed moderate mortality rates against *D. suzukii*, suggesting potential for integrated pest management. Unexpectedly, high dynamized dilutions (HDDs) increased *D. suzukii* consumption and oviposition, indicating potential for trap enhancement rather than direct pest control. Future research should assess these compounds' effects on beneficial insects and explore HDDs as attractants for improved monitoring and trapping. Recommendations emphasize vineyard monitoring during grape ripening stages, aligning with peak of *D. suzukii*. Cultivar susceptibility findings enable targeted pest management strategies, optimizing control efforts. Overall, this research provides

information that can be integrated in a management program for D. suzukii in vineyards located in the São Joaquim region, Santa Catarina, Brazil.
Keywords: Integrated pest management; Biological control; Spotted-wing Drosophila; Fruit production.

RESUMO

BRILINGER, D. **Manejo integrado de *Drosophila suzukii*: monitoramento e estratégias de manejo**. 2024. 166 f. Tese (Doutorado em Produção Vegetal) – Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Programa de Pós-Graduação em Produção Vegetal, Lages. 2024.

A mosca da fruta invasora, *Drosophila suzukii*, tornou-se uma ameaça global à produção de pequenas frutas devido à sua expansão global e características biológicas. Sua capacidade de produzir morfotipos sazonais permite-lhe sobreviver em diferentes condições climaticas, resultando em múltiplas gerações por ano. As fêmeas preferem frutas de casca fina para oviposição. Após a postura dos ovos, as larvas eclodem e consomem a polpa da fruta causando perdas econômicas aos produtores. Além disso, os pequenos ferimentos oriundos da oviposição facilitam a penetração de microorganismos patogênicos. Em países como os Países Baixos e Brasil, já foram registradas perdas significativas devido aos danos de *D. suzukii* em culturas como pequenas frutas e uvas. Devido a isso, esta pesquisa explorou diferentes abordagens para o manejo integrado de pragas para contribuir no desenvolvimento de um programa de manejo para *D. suzukii*, com foco em vinhedos em São Joaquim, Santa Catarina, Brasil. O monitoramento de campo revelou densidades elevadas de capturas de *D. suzukii* nas bordas das florestas em comparação com os vinhedos, com migração de fêmeas para os vinhedos durante o período de maturação das uvas. Variáveis climáticas, particularmente a umidade relativa, influenciaram significativamente nas capturas de *D. suzukii*, variando de acordo com o microclima em cada vinhedo. Avaliações laboratoriais destacaram a suscetibilidade de cultivares de uva à *D. suzukii*, enfatizando a importância da integridade da casca e da escolha de oviposição. Características físico-químicas das uvas, como resistência da casca e pH, impactam os comportamentos de oviposição de fêmeas de *D. suzukii*. Compostos botânicos como matrine e oximatrine causaram mortalidade de adultos de *D. suzukii*, sugerindo potencial para o manejo integrado de pragas. Altas diluições dinamizadas (ADDs) aumentaram o consumo e a oviposição de fêmeas de *D. suzukii*, indicando potencial para exploração como aditivos em armadilhas, ao invés de controle direto de pragas. Pesquisas futuras devem avaliar os

efeitos desses compostos botânicos em insetos benéficos e explorar as ADDs como atrativos para melhorar o monitoramento e as armadilhas. Recomendações enfatizam o monitoramento dos vinhedos durante o período de maturação das uvas, momento em que as fêmeas tendem a migrar para o vinhedo. Os resultados sobre a suscetibilidade das cultivares de uva permitem o direcionamento de estratégias de manejo, otimizando os recursos de controle. No geral, esta pesquisa fornece informações que podem ser integradas a um programa de manejo para *D. suzukii* em vinhas localizadas na região de São Joaquim, Santa Catarina, Brasil.
Palavras-chave: Manejo Integrado de Pragas; Controle sustentável; Drosófila da asa-manchada; Fruticultura.

SAMENVATTING

De invasieve fruitvlieg, *Drosophila suzukii*, is een bedreiging geworden voor de productie van zachtfruit vanwege zijn wereldwijde verspreiding en biologische kenmerken. Het vermogen om seizoensgebonden morfologisch verschillende vormen te produceren stelt deze soort in staat te overleven in verschillende klimaten, wat resulteert in meerdere generaties per jaar. Vrouwtjes geven de voorkeur aan zachtfruit voor het leggen van eieren, wat economische verliezen veroorzaakt doordat de larven zich voeden met vruchtvlees, en openingen creëren voor pathogene micro-organismen. In landen zoals Nederland en Brazilië zijn significante fruitverliezen geregistreerd, waarbij gewassen zoals druiven worden aangetast. Dit onderzoek verkent verschillende benaderingen voor geïntegreerd plaagbeheer en draagt bij aan de ontwikkeling van een geïntegreerd beheerprogramma voor *D. suzukii*, specifiek voor wijngaarden in de regio São Joaquim, Santa Catarina, Brazilië. Veldwerkmonitoring onthulde hogere plaagdichtheden aan de bosranden in vergelijking met wijngaarden, waarbij migratie naar wijngaarden plaatsvond tijdens de druivenrijping. Klimaatvariabelen, met name de relatieve luchtvochtigheid, hadden een significante invloed op de vangsten van *D. suzukii*, die per microklimaat in elke wijngaard varieerden. Laboratoriumexperimenten benadrukten dat druivensoorten verschillen in de vatbaarheid voor *D. suzukii*, twee grote factoren hierin zijn de integriteit van de schil en de keuze voor het leggen van eieren. Fysische en chemische eigenschappen van druiven, zoals huidweerstand en pH, beïnvloeden het eileggedrag van *D. suzukii*. Botanische stoffen zoals matrine en oxymatrine resulteerden in gematigde sterftecijfers in *D. suzukii*, wat potentieel laat zien voor het gebruik in geïntegreerd plaagbeheer. Tegen de verwachting in verhoogden hoge dynamische verdunningen (HDV's) de consumptie en het leggen van eieren in *D. suzukii*, en kan mogelijk worden gebruikt als lokmiddel voor het verbeteren van vallen in plaats van te worden gebruikt als directe plaagbestrijding. Toekomstig onderzoek moet de effecten van deze stoffen op nuttige insecten beoordelen en HDV's verkennen als lokmiddel voor verbeterde monitoring en vangst. Aanbevelingen benadrukken het belang van het monitoren van wijngaarden tijdens de druivenrijpingsfase, wat samenvalt met de piekactiviteit van *D. suzukii*. De bevindingen over de vatbaarheid van druivensoorten maken het mogelijk om gerichte plaagbeheerstrategieën te ontwerpen, waardoor de

bestrijdingsinspanningen kunnen worden geoptimaliseerd. Resultaten uit dit onderzoek kunnen worden geïntegreerd in de ontwikkeling van een bestrijdingsprogramma voor *D. suzukii* in wijngaarden gelegen in de regio São Joaquim, Santa Catarina, Brazilië
Keywords: Geïntegreerd plaagbeheer; Biologische bestrijding; Suzuki-fruitvlieg; Fruitproductie.

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1 GENERAL INTRODUCTION

1.1 *Drosophila suzukii* AS A GLOBAL FRUIT PEST IN THE WORLD

Since the beginning of agriculture, the commercialization of agricultural products such as grains, seeds, fruits, and vegetables, has required the exchange and transport of these products. This has played an essential role in the development of communities and societies all around the world (Ramankutty *et al.*, 2018). Nowadays, with the globalization process, these trade and transportation activities have assumed international proportions (Köhler, 2014). Perishable products have notably benefited from improvements in agriculture systems and their associated technologies, facilitating the exportation of products like fruits across long distances (Faqeerzada *et al.*, 2018). This, in turn, has not only reinforced international commerce but also contributed to increased income for countries engaged in agricultural business.

On the other hand, globalization and its transportation activities have given rise to a range of challenges, affecting not only agriculture but also human health, as it has enabled the global exchange of pests and diseases (Marsden; Sonnino, 2012; Ramankutty *et al.*, 2018). This explains why nowadays in agriculture we are facing many exotic pests causing damage to agricultural crops and leading to substantial economic losses. An example is the impact of *Drosophila suzukii* (Diptera: Drosophilidae) on fruit production. The species *D. suzukii* is originally from Asia and spread across the world in the past 1.5 decade, probably due to fruit trade across seas (Berry, 2020). This invasive species, commonly known worldwide as the spotted-wing drosophila (SWD), owes its name to the distinctive black spots on the wings of males (Figure 1). Males can also be distinguished from closely related species by the two small rows of 3–4 peg-like setae on their protarsus, forming a sex comb (Yuzuki; Tidon, 2020). In contrast, females do not have spots on their wings and are identified by having a large serrated ovipositor (Yuzuki; Tidon, 2020).

Described by Matsumura in 1931 from Japan (Kanzawa, 1936) as well as China, Korea, and Thailand, *D. suzukii* was recorded for the first time as an invasive pest in Hawaii in 1980, at the same time with three other *Drosophila* species (O'Grady, Beardsley, Perreira, 2002). The records of *D. suzukii* mention it was found in highlands, mostly in pristine rainforests, suggesting its high potential to disperse (Hauser, 2011; O'Grady *et al*., 2002).

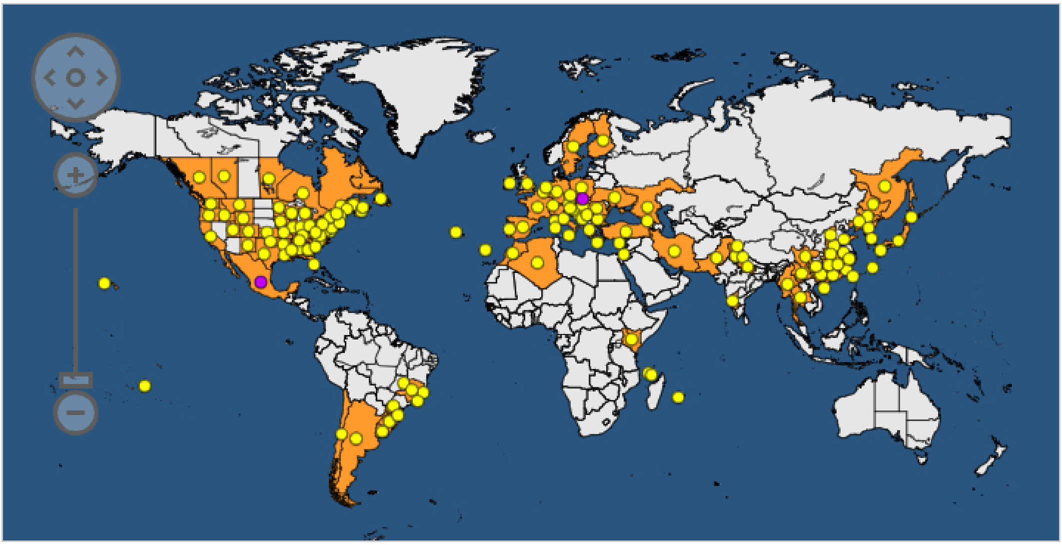
Figure 1 - Adults of *Drosophila suzukii*. Male specimen (left) and female (right).

Source: McEvey, 2017 (https://extension.usu.edu/pests/research/spotted-wing-drosophila).

In Europe and North America, it has been recorded since 2008, probably through separate introductions from Southeast Asia (Fraimout *et al.*, 2017). From there, it spread to South America in 2013, and Africa in 2017 (Boughdad *et al.*, 2021; Calabria *et al.*, 2012; Deprá *et al.*, 2014; Lee *et al.*, 2011) (Figure 2). Predictions show that *D. suzukii* has the potential to expand globally even further, also invading other areas such as Australia and New Zealand (Ørsted; Ørsted, 2019; Santos *et al.*, 2017).

Since the beginning of the invasion of *D. suzukii* in the United States (U.S.) and Europe, reports about economic losses due to the damages caused by this pest have been published. In 2008, the states of California, Oregon, and Washington in the U.S.A., reported economic losses ranging from 33% to 50% for berry crops, based on the maximum reported yield losses due to *D. suzukii* (Bolda; Goodhue; Zalom, 2010). Just one year later, in 2009, the estimates of yield losses had increased up to 80%. Considering a 20% yield loss for strawberries, blueberries, raspberries, and cherries, in these three states, it could be turned into US$511 million of production losses (Walsh *et al.*, 2011).

Figure 2 - Worldwide *Drosophila suzukii* distribution map, based on records until October 2023 in EPPO database (https://gd.eppo.int/taxon/DROSSU/distribution). Countries and provinces in orange and yellow dots are locations with confirmed presence of the *D. suzukii*

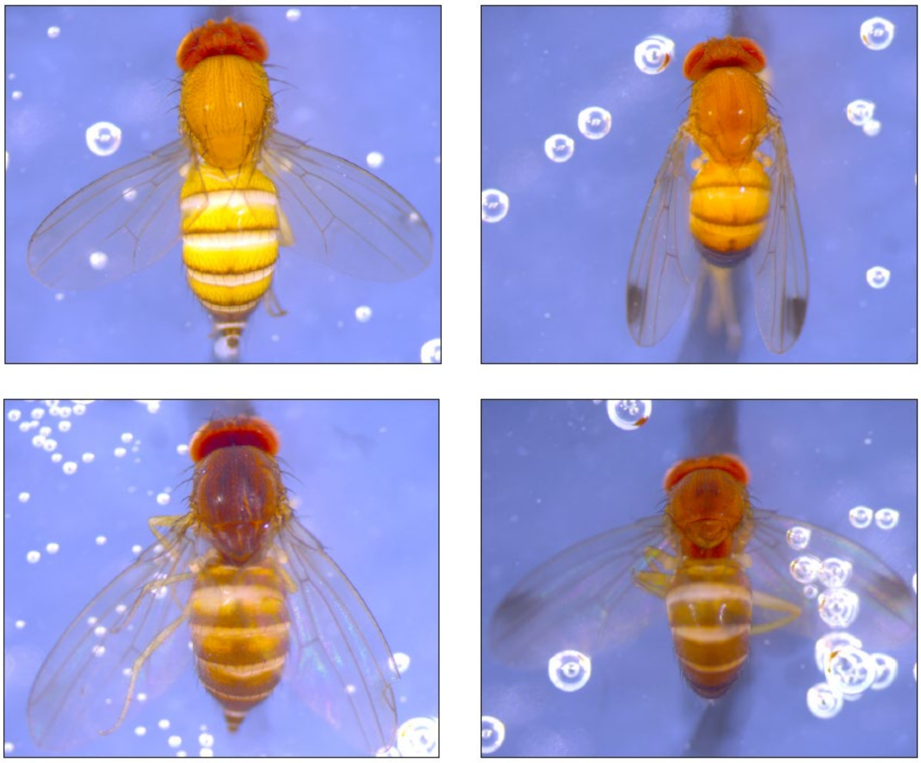


It is reported that from 2009 to 2014 the raspberry industry in California, U.S.A., faced approximately US$ 39.8 million in economic losses due to *D. suzukii* damage (Farnsworth *et al.*, 2017). In Minnesota, yield losses of approximately 159 thousand kg were quantified in conventional raspberry production in 2017, which corresponds an approximately US$2.36 million of economic loss (Digiacomo *et al.*, 2019). In Europe, *D. suzukii* damage caused losses of up to 80% in strawberries in France; in Italy, harvest losses were around 30 to 40% in blueberries, blackberries, and raspberries (Lee *et al.*, 2011). In Trentino, a region of Italy, the total damage to berry crops could be valued at more than 3 million Euros in 2011 (De Ros *et al.*, 2013).

Biological aspects of *Drosophila suzukii*

The global expansion and distribution of *D. suzukii* can be attributed to its biological and ecological characteristics, such as adaptive phenotypic plasticity that allows it to cope with a wide range of climate conditions, high fecundity as well as its wide host range (Tait *et al.*, 2021). Different environments and climate conditions can affect the *D. suzukii* population stage-structure (Wiman *et al.*, 2014). Adults of *D. suzukii* develop at temperatures from 10 ºC to 30 ºC, with optimal developmental conditions between 20 ºC and 25 ºC (Kanzawa, 1939). This species exhibits a remarkable ability to pass through winter periods with sub-zero temperatures, adjusting to seasonal climate conditions through phenotypic plasticity in physiological and morphological traits (Hamby *et al.*, 2016; Stephens *et al.*, 2015). The adults of *D. suzukii* manifest phenotypic plasticity in producing two seasonal morphotypes, winter morphs (WM) and summer morphs (SM). These morphotypes differ in gene expression and metabolic profile (Shearer *et al.*, 2016). The winter morph presents darker body pigmentation and longer wings than summer morphs (Figure 3).

Figure 3 - The *Drosophila suzukii* morphotypes: summer morph adults (top panels) and winter morph (bottom panels)

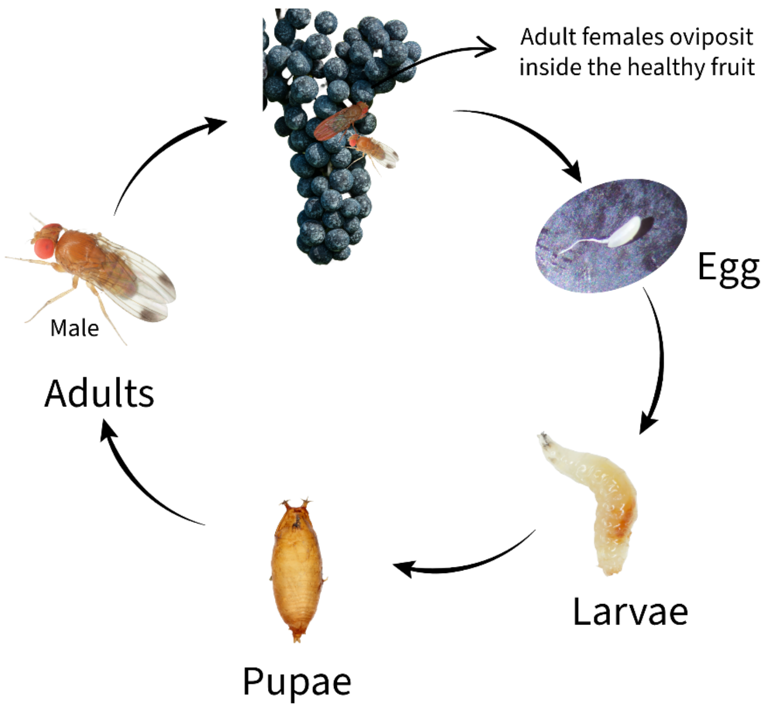


Source: Panel (2021).

It also has increased cold-tolerance ability and can enter a reproductive diapause when the temperature is below 5 °C (Funes *et al.*, 2018; Jakobs; Gariepy; Sinclair, 2015; Stephens *et al.*, 2015). Female WM that are mated in autumn and overwintered, may show mature eggs by early spring, ready to infest the earliest ripening commercial fruits such as cherries, and other suitable non-crop fruits (Panel *et al.*, 2018). This reproductive strategy contributes to the emergence of the first generation of summer morphotypes, bolstering the pest population during late spring (Panel *et al.*, 2018).

Combined with these characteristics, *D. suzukii* is a *r*-strategist, showing a high fecundity, fast sexual ripening, early start of reproduction, production of a great number of offspring per individual, and short generation times (Garcia, 2020). The biological cycle of *D. suzukii* from egg to adult (Figure 4) lasts an average of 9 to 11 days in thermal conditions of 25ºC (Kanzawa, 1939).

Figure 4 - The *Drosophila suzukii* life cycle in a grape berry as an example of a fruit host



Source: Author (2024).

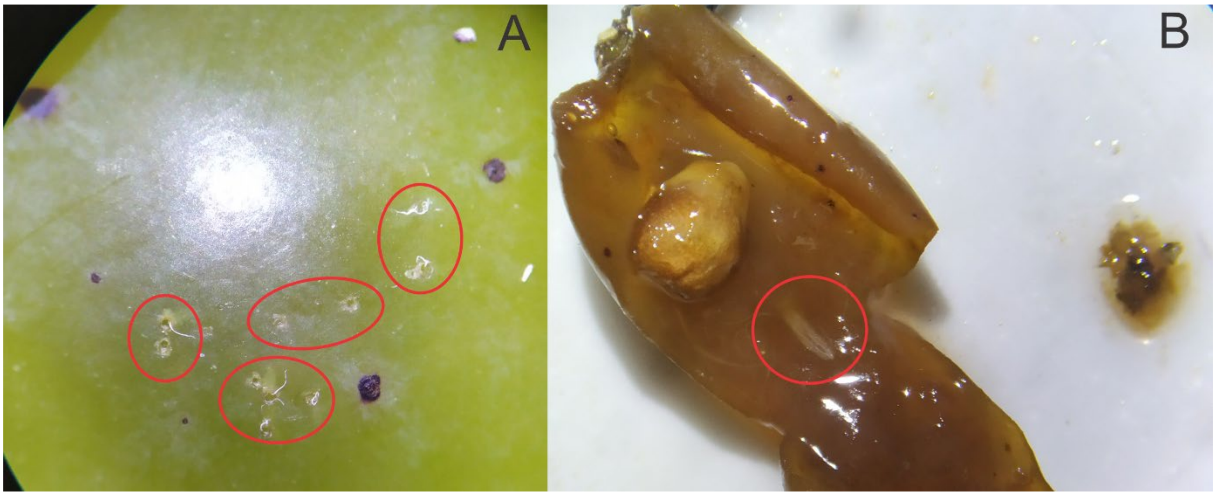
The adults may reach their maturity one or two days after emergence, depending on weather conditions, and females may start to lay eggs when two days old (Cini; Ioriatti; Anfora, 2012). Females may lay 1 to 3 eggs per oviposition site and 7 to 16 eggs per day. Considering that the females may oviposit for 10-59 days, they can lay up around 400 eggs during their life, depending on the nutrition and temperature conditions (Cini *et al.* 2012; Kanzawa 1939). The eggs are white and have two thin external filaments, used for transpiration. The egg period lasts from 1 to 2 days in temperature between 20–25°C varying according to the host (Walsh *et al.*, 2011). The larvae have a milky-white color and cylindrical shape and go through three larval instars inside the fruit, maturing even in 5 days in temperatures between 20–25°C (Walsh *et al.*, 2011). The pupal phase occurs inside the fruit as well close to the surface, and less frequently, in the soil, with a duration of 6 to 10 days in temperature between 20–25°C varying according to the host (Walsh *et al.*, 2011). Adult lifespans can be different depending on the morphotype. The Winter morphotype can live around 5 months in temperature conditions of 10 °C, while the Summer morphotype can live around 7 months in temperature conditions of 20 °C (Panel *et al.*, 2020). Additionally, its short generation implies several complete generations in a single cropping cycle and up to 7 to 15 generations in a year (Cini; Ioriatti; Anfora, 2012; Funes *et al.*, 2018).

Drosophila suzukii is a polyphagous insect with an extensive host range. It prefers fruits with soft skin, where it oviposits just underneath the fruit skin, e.g., berries and cherries (Bellamy; Sisterson; Walse, 2013; Burrack *et al.*, 2013). In Asia, researchers have identified 58 plant species from 11 families as *D. suzukii* hosts, including non-crop fruit species (Kirschbaum *et al*., 2020). In North America 85 crop and non-crop fruit species have been documented as *D. suzukii* hosts, while Latin America has reported 65 plant species hosting this pest (Garcia *et al*., 2022; Kirschbaum *et al*., 2020). In Europe the scenario is even worse, presenting 126 plant species as *D. suzukii* hosts (Kirschbaum *et al*., 2020). This also includes many non-crop hosts in natural habitats, but also in gardens or parks. These ecosystems contribute to the *D. suzukii* population maintenance, providing winter morph survivors to use early-spring fruit for food and reproduction (Grassi *et al.*, 2018; Kenis *et al.*, 2016; Manko; de Figueroa; Oboňa, 2021). Moreover, *D. suzukii* possibly can use mushrooms and bird manure as a protein source during the overwintering period, although this remains somewhat controversial (Stockton *et al.*, 2019).

The bioecological characteristics of *D. suzukii* have facilitated its global expansion and also explain why this species poses a significant threat to fruit production (Cini; Ioriatti; Anfora, 2012). Most species within the Drosophilidae family cannot puncture the skin of fruit and primarily feed on microorganisms associated with fruit decay; they are generally not regarded as agricultural pests (Carson, 1971). In contrast, *D. suzukii* distinguishes itself with its serrated ovipositor that enables the female to deposit its eggs in ripe fruits (Figure 5A), causing damage that results in substantial economic losses for fruit growers (Funes *et al.*, 2018; Walsh *et al.*, 2011). The main damage of *D. suzukii* is caused by larvae that feed on the fruit pulp (Figure

5B) (Mazzetto *et al.*, 2015). Also, the small perforations resulting from egg-laying favor secondary infections by the penetration of phytopathogenic microorganisms contributing to the rapid deterioration of the fruit. In addition, the exudation of small portions of juice due to the punctured fruit skin can attract other insects that can cause damage and accelerate fruit decay (Ioriatti *et al.*, 2015).

Figure 5 - Damage caused by *Drosophila suzukii* female's oviposition in white grape (A). Larvae of Drosophila suzukii consuming pulp of white grape (B).



Source: Author (2021).

1.2 SPOTTED-WING DROSOPHILA IN THE NETHERLANDS AND BRAZIL

In the Netherlands, monitoring research was set up between 2007 and 2009 to assess whether *D. suzukii* was present in the Netherlands. Traps containing attractive bait were distributed in a public garden, the Noorderplantsoen in the Groningen province, in the north of the Netherlands. However, no *D. suzukii* adults were captured (Calabria *et al.*, 2012). The first record of *D. suzukii* was in 2012 (Asplen *et al.*, 2015), and the Dutch Fruit Growers Organization suspects that economic damage caused by *D. suzukii* occurred as early as 2013, with an estimative of fruit loss worth 10 million euros (Helsen; van Bruchem; Potting, 2013; Nijland; Helsen, 2015). Adults of *D. suzukii* were captured in cherry orchards, vineyards, and gardens from 2016 to 2017 in sites near Wageningen, and abandoned or untreated cherry trees may present the first heavy infestations in these sites (Kenis *et al.*, 2016; Panel *et al.*, 2018). Since its start, the monitoring surveys carried out in the Netherlands showed that the species *D.*

suzukii was identified as infesting fruit of 52 plant species (Kenis *et al.*, 2016). Non-crop plants such as *Skimmia japonica*, *Aucuba japonica*, *Elaeagnus* x *ebbingei*, and *Viscum album* were identified as early spring host plants of *D. suzukii* (Panel *et al.*, 2018).

In Brazil, the first record of *D. suzukii* was in 2013, and since then, the species has already been reported in eight states (Deprá *et al.*, 2014; Garcia *et al.*, 2022). The records of *D. suzukii* range from presence in peach orchards and vineyards to infesting fruits such as strawberry, blueberry, feijoa, papaya, guava, blackberry, and pitanga (Brilinger *et al.*, 2023; Foppa *et al.*, 2018; Souza *et al.*, 2017; Vilela; Mori, 2014; Wollmann *et al.*, 2020; Zanuncio-Junior *et al.*, 2018). The presence of *D. suzukii* was also reported from an environmental protection area (Paula, Lopes, Tidon, 2014). Damages from *D. suzukii* infestation were recorded in more than 30% of the commercial strawberry crops in 2014, and the potential losses estimated for peach and fig fruit might be 20% and 30%, respectively (Benito; Lopes-da-Silva; Santos, 2016; Santos, 2014). To forecast the potential loss of fruit production in Brazil due to the *D. suzukii* attack, a model was used to predict the *D. suzukii* distribution across the country, based on its temperature requirements. This model predicts that most of the territory is not favorable to its development (Benito; Lopes-da-Silva; Santos, 2016). However, in the states of the southern region, climatic conditions are considered favorable to highly favorable to the presence and development of *D. suzukii* (Benito; Lopes-da-Silva; Santos, 2016).

The southern region of Brazil concentrates 73% of Brazilian grape production, mostly on small properties with distinct regional characteristics (Kist *et al.*, 2018). The highland area of Santa Catarina state, with altitudes between 900 and 1400 m, has stood out in grape vineyards for the production of wines (Cazella; Souza; Nunes, 2019; Tonietto; Sotés Ruiz; Gómez-Miguel, 2012). No official records of the presence of *D. suzukii* were reported for the southern region, until 2018 (Brilinger *et al.*, 2023). However, in the 2014/15 season, an infestation of *D. suzukii* was observed in Cabernet sauvignon grapes (Arioli; Botton; Bernardi, 2015). Furthermore, there were reports from winemakers who observed drosophilid larvae during the winemaking process of Merlot and Sangiovese grapes in the same crop season. Additionally, technicians and farmers reported an increase in pre-harvest diseases, which might be associated with the *D. suzukii* infestation, as the oviposition punctures made by females can damage the grapes and facilitate the penetration of pathogens (Ioriatti *et al.*, 2018). This suggests that from 2014 onwards, *D. suzukii* has become established in this southern region of Brazil.

1.3 THE IMPORTANCE OF DEVELOPING AN INTEGRATED MANAGEMENT FOR *Drosophila suzukii*

The economic loss in soft-fruit crops due to *D. suzukii* damage can be high, as stated before. Therefore, there is a growing focus on assessing the economic impact of prevention and control measures for *D. suzukii*. Preventing *D. suzukii* infestations leads to an increase in costs of production, including labor and chemical products for monitoring and control. Additionally, *D. suzukii* damage/infestations can result in the loss of foreign markets for fruit trade, mainly where this species has not been recorded yet (Cini *et al.*, 2014; Farnsworth *et al.*, 2017; Lee *et al.*, 2011). The benefits of investing in the management of *D. suzukii* far outweigh the costs of not controlling this species. Studies have shown that adopting integrated *D. suzukii* management practices can reduce potential economic losses from 13% to 7% (De Ros *et al.*, 2015; Goodhue *et al.*, 2011).

In general, Integrated Pest Management (IPM) programs seek to create synergies by integrating many possible and different methods, combining a wide range of approaches, to manage insect pests in crop systems (Barzman *et al.*, 2015). These techniques can range from cultural, physical, behavioral, and biological methods to chemical intervention when necessary (Schetelig *et al.*, 2018). Since *D. suzukii* emerged as a pest, a wide range of techniques has been explored for its management (Tait *et al.*, 2021). For the effectiveness of IPM, however, it is crucial to recognize the importance of adapting approaches to specific regions and crops (Schetelig *et al.*, 2018; Tait *et al.*, 2021). It may be impossible to identify a single optimal management strategy for *D. suzukii* that applies to the wide variety of production systems that are affected by this pest.

Taking into account the sustainable strategies to be applied in IPM, the use of natural substances needs to be considered (Schetelig *et al.*, 2018). Several natural compounds were identified as acting as repellents, toxicants, fumigants, ovicides, or oviposition deterrents (Dam; Molitor; Beyer, 2019). Additionally, the use of botanical compounds as insecticides remains an area with significant potential but requires further studies. Another less-explored technique for managing agricultural pests is the use of high-dynamized dilutions (HDD). This is a serial dilution of compounds (e.g., minerals, plants, or animal extracts), which is vigorously shaken at each dilution step to better extract and uniformly mix the substance. The use of high-dynamizes dilutions has the potential to reduce or minimize the damage caused by pests and diseases in crops without causing toxicity to plants, animals, or humans, but rather through the restoration of the vital energy balance of the organisms that cohabit into the environment (Boff; Verdi; Faedo, 2021).

Before implementing any management technique, however, it is necessary to perform monitoring, which is considered the basis of IPM (Barzman *et al.*, 2015). Monitoring enables estimating population densities and dynamics, and it indicates the relative pest pressure in the field; this should form the basis for decisions regarding *D. suzukii* control (Tochen *et al.*, 2014). Monitoring should not be limited to the orchard; forest borders should also be monitored, as these environments can host *D. suzukii* adults (Briem *et al.*, 2018). In addition to monitoring, knowledge of fruit susceptibility has become a valuable tool for farmers. This information helps them identify which cultivars require more attention, and which may be sufficiently protected with reduced chemical treatments against *D. suzukii* (Tait *et al*., 2021).

1.4 OBJECTIVE AND THESIS OUTLINE

The establishment of *Drosophila suzukii* in Brazilian vineyards, particularly in the highland region of Santa Catarina state, highlights the necessity of research, both in the field and the laboratory, to improve the development of integrated *D. suzukii* management. The key step in this process involves gaining an understanding of the *D. suzukii* behavior in the field, as well as identifying the specific climatic conditions that drive population growth in this region. Simultaneously, the development of sustainable tools to control *D. suzukii* is essential. Thus, the objective of the research presented in this thesis was to monitor *D. suzukii* in Cabernet Sauvignon vineyards located in the highland region of Santa Catarina State, Brazil, and investigate sustainable strategies to become useful to be applied to a *D. suzukii* integrated management program. The results presented in this thesis are from research developed both in Brazil and the

Netherlands as part of the double degree agreement between the Santa Catarina State University (BR) and the University of Groningen (NL).

Two research chapters of this thesis report on research that was carried out in the field in the highland region of Santa Catarina State, Brazil. **Chapters II and III** report on research to monitor the *Drosophila suzukii* population dynamics in vineyards in the Santa Caterina State. The research in **Chapter II** was carried on in four commercial vineyards of Cabernet Sauvignon, over the course of two years. **Chapter III**, on the other hand, presents research carried out in an experimental vineyard, over three grape seasons. The monitoring was carried out both inside the vineyards and in the surrounding forest borders, allowing us to gain insights into the role of these habitats on *D. suzukii* population dynamics. Traps, baited with an attractive lure, were sampled weekly, and the captured insects were counted and identified. Furthermore, climatic data was collected weekly, to identify the specific climate conditions that most significantly impact the *D. suzukii* population dynamics in vineyards in the highland region of Santa Catarina state, Brazil. The relation between population fluctuations and climate variables was analyzed with multiple regression models.

The research presented in **Chapter III** was also aimed at evaluating the susceptibility of the grape wine cultivars that hold economic significance, such as Cabernet Sauvignon, Cabernet Franc, Sangiovese, Chardonnay, and Sauvignon Blanc. The research was conducted in an experimental vineyard, composed of 54 grape wine cultivars (germplasm bank *in vivo*). The susceptibility of grape wine cultivars was evaluated to provide information on which grape wine cultivars are most at risk for becoming infested with *D. suzukii.* This could inform farmers on the cultivars that they should pay more attention to during the ripening period. This awareness could help avoid high levels of infestation yet reduce the economic and environmental impact of the application of chemical pest control. Together with the monitoring, this information can enable the application of techniques to control *D. suzukii* when it is necessary.

The data reported in **Chapter IV** aimed to further evaluate the susceptibility of different grape wine cultivars to *D. suzukii*, in a laboratory setting. The susceptibility was assessed for the red cultivars Cabernet Sauvignon, Cabernet Franc, Merlot, Sangiovese, and Montepulciano and white cultivars Chardonnay and Sauvignon Blanc. In a behavioral no-choice assay, females were provided with undamaged and damaged grapes of each cultivar, to assess their oviposition in these grapes. In parallel with the susceptibility bioassays, the physical (penetration resistance and color patterns) and chemical (soluble solids content (°Brix), total titratable acidity (TTA), and pH) characteristics for each grape cultivar were evaluated. The relation between the susceptibility and physical-chemical characteristics was analyzed. This information can help in *D. suzukii* management, by identifying which cultivar characteristics diminish and/or favor *D. suzukii* oviposition, to also predict risk for infestation in the field.

The other data chapters report on research that was conducted in the laboratory, both in Brazil and the Netherlands. To explore alternative and more sustainable tools to be applied in *D. suzukii* management, we assessed the effectiveness of a new biorational insecticide and the high dynamized dilutions for inducing mortality or reducing reproduction. The research in **Chapter V** aimed to evaluate the effectiveness of the botanical compounds matrine and oxymatrine to induce mortality in adults of *D. suzukii* in feeding assays. These botanical compounds are derived from the roots of the plant *Sophora flavences* and have shown potential as insecticides against various agricultural pests. However, they had not been explored against Diptera yet. The botanical compound matrine is available as a commercial insecticide, registered as Matrine®, and it was used to evaluate the potential of this insecticide against *D. suzukii*. Adult mortality was measured in feeding assays. The insecticide was offered in droplets, deposited on small plastic plates inside the test cages. To further characterize the effectiveness of this compound, we also assessed the consumption rate and mortality of the closely-related botanical compound oxymatrine using a Capillary Feeder (CAFE) assay.

In **Chapter VI**, the aim was to assess the effect of high dynamized dilution (HDD) on the feeding and reproduction of *Drosophila suzukii*, both in parental adults and its progeny. As this approach is relatively underexplored in pest management, we developed and adapted methodologies to investigate high dynamized dilution effects. Based on the literature, four selected HDDs (*Lilium tigrinum*, *Bufo rana*, *Hyosciamus niger*, and *Sepia succus*) were prepared, and another one was made using crushed *D. suzukii* adults (*D. suzukii* nosode). For the evaluation of feeding behavior, consumption rates were measured using the CAFE assay. To evaluate the impact on reproduction, HDD was incorporated into the larval diets. Reproduction was quantified by counting the number of eggs and emerged adults.

In the final chapter of this thesis, **Chapter VII**, I present a comprehensive discussion of my research, to outline an integrated management approach for *Drosophila suzukii*. This discussion summarizes the research findings presented in this thesis and integrates them with other studies and the existing knowledge that complement our understanding of the strategies for *D. suzukii* management. Additionally, it highlights promising directions for future research on management and *D. suzukii* control.

2 *Drosophila suzukii* (DIPTERA: DROSOPHILIDAE): AN EXOTIC PEST OF SMALL FRUITS ESTABLISHED IN CABERNET SAUVIGNON VINEYARDS OF SANTA CATARINA STATE, BRAZIL

2.1 ABSTRACT

To characterize the annual *Drosophila suzukii* population dynamics in vineyards in Santa Catarina, Brazil, and to relate it to the local climatic conditions, we conducted a weekly monitoring survey in four commercial vineyards of Cabernet Sauvignon, between November 2018 and October 2020. The *D. suzukii* population was monitored both inside the vineyards and the surrounding forest border and correlated with climate variables. To capture the insects, plastic pot traps baited with the attractive Droskidrink were used. The traps were sampled weekly throughout two years, and the captured insects were counted and identified. The relation between population fluctuations and climate variables was analyzed with multiple regression models. The numbers of *D. suzukii* captured in the forest borders were typically much higher than those into the vineyards, but in certain periods of the year, *D. suzukii* was indeed detected inside all vineyards. The number of captures varied between the years of monitoring, where the first year had higher numbers of adults captured for all monitored sites. The *D. suzukii* population numbers in all monitored vineyards were positively correlated with high relative humidity. The results show that there is indeed a significant risk of *D. suzukii* infestations in vineyards, but only during a distinct period during the ripening of the grapes. We recommend that farmers specifically perform *D. suzukii* management in their vineyards during this relatively short period of the year.
Keywords: monitoring, spotted-wing-drosophila, *Vitis vinifera*.

2.2 INTRODUCTION

Viticulture is one of the most representative sectors in job and income generation in Brazil (Carvalho; Kist; Beling, 2020), where 73% of the total grape production occurs on small properties with distinct regional characteristics (Kist *et al.*, 2018). São Joaquim is a municipality located in Santa Catarina State, and stands out in the production of wine grapes, due to the fact that it has the largest number of vineyards and, consequently, the largest area cultivated with wine grapes (168.13 ha) (Vianna *et al.*, 2016). Every year during the summer season, farmers face several obstacles during grape production, such as pests and pathogens. The presence of *D. suzukii* larvae in wine grapes has been documented in other countries, resulting in adverse effects such as cracking, diseases, and attraction of other animals, including wasps, bees, and birds, particularly during the harvest period (Atallah *et al.*, 2014; Ioriatti *et al.*, 2015).

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) is an exotic and polyphagous species, considered a pest, especially in temperate countries (Walsh *et al.*, 2011). Females of *D. suzukii* can infest and cause damage to fruits in ornamental, wild, and economically important fruit trees (Mitsui; Beppu; Kimura, 2010; Poyet *et al.*, 2014). In Brazil, during the past decade, *D. suzukii* has already been detected in southern and southeastern regions, in peach orchards (*Prunus persica*) (Foppa *et al.*, 2018), apple (*Malus domestica*) (Oliveira; Amaral Neto; Santos, 2015) and vineyards (*Vitis vinifera*) (Brilinger *et al.*, 2021). It was also identified infesting strawberry fruits (*Fragaria* x *ananassa*) (Santos, 2014; Wollmann *et al.*, 2020), blueberry (*Vaccinium myrtillus*) (Vilela; Mori, 2014), feijoa (*Acca sellowiana*) (Souza *et al.*, 2017), papaya (*Carica papaya*), guava (*Psidium cattleianum*) (Wollmann *et al.*, 2020; Zanuncio-Junior *et al.*, 2018), blackberry (*Rubus* spp.) and pitanga (*Eugenia uniflora*) (Wollmann *et al.*, 2020). In the midwest region of Brazil, in the Federal District, *D. suzukii* was identified in traps placed in an environmental protection area (Paula, Lopes, Tidon, 2014).

The population dynamic of *D. suzukii* shows clear seasonality. Populations decline during winter, and build up over the course of spring, summer, and autumn (Rossi-Stacconi *et al.*, 2016). The adults can track the succession of ripening fruits in various cropping systems. Also, the surrounding area around vineyards may affect the infestation risk, as alternative fruit hosts can play an important role for the population dynamics of *D. suzukii*. Infestations may occur earlier in orchards surrounded by alternative hosts than in orchards where there are no alternative hosts (Weißinger *et al.*, 2019). Understanding the *D. suzukii* population dynamics in orchard borders and other environments is important to verify their behavior and their possible impacts on fruit quality (Deprá *et al.*, 2014).

Using climex models based on information from the ecoclimate index, Benito *et al*. (2016) reported that the southern region of Brazil, where more than 90% of the vine grape production comes from, is considered favorable to highly favorable to *D. suzukii* development and establishment. Indeed, in vineyards of Cabernet Sauvignon, Merlot, and Sangiovese grapes, located in the municipality of São Joaquim, SC, *D. suzukii* infestation was detected during the vinification process by the presence of larvae into the grape juice (Arioli; Botton; Bernardi, 2015). Furthermore, technicians responsible for the production of wineries reported an increase in the incidence of pre-harvest diseases, which may be related to the *D. suzukii* damage, since its oviposition punctures of the grapes' skin facilitate the penetration of pathogens. Considering the high potential of economic losses that the damage of *D. suzukii* can cause, and the confirmation of its presence in the São Joaquim municipality where wine grapes are in full expansion of cultivation, it is essential to know the population dynamics of this pest.

The objective of this study was to evaluate the annual population dynamics of *D. suzukii* in vineyards in the municipality of São Joaquim, in Santa Catarina State. The *D. suzukii* population dynamics were monitored in four commercial Cabernet Sauvignon vineyards, as well as the forest border around the vineyards, over two years. The weekly numbers of flies that were captured in traps were related with climatic variables. By analysing the relationship between the population dynamics and the climatic variables, we hope to provide the farmer with information to predict the increase and decrease of the pests' population and improve management strategies against *D. suzukii* in vineyards in the southern region of Brazil.

2.3 MATERIAL AND METHODS

2.3.1 Study area and period

The study was conducted from November 2018 to October 2020 in four distinct commercial Cabernet Sauvignon vineyards located in São Joaquim, Santa Catarina, Brazil, identified as Sites A, B, C, and D (Table 1). These vineyards were situated at varying distances from each other, ranging from 2 to 10 km. In all cases, the vine plants were cultivated using an espalier system. The vineyard's borders are composed of native characteristic vegetation of the Alto-Montana Mixed Ombrophylous Forest (FOM) type, in which Myrtaceae species are predominant (Higuchi *et al.*, 2013). Next to the vineyards, it is also possible to find orchards of commercial fruit such as apples, which are also part of the agricultural activity in the municipality of São Joaquim, SC.

Table 1 - Characterization of Cabernet Sauvignon vineyards where *Drosophila suzukii* was monitored. São Joaquim, SC, Brazil

Site	Location	Area (ha)	Planting*	Altitude (m)
A	28°16'29.27"S 49°56'13.48"O	1.10	2007	1170
B	28°16'11.14"S 49°58'10.97"O	1.67	2003	1280
C	28°15'34.26"S 49°57'34.75"O	4.78	2002	1280
D	28°13'47.95"S 49°59'05.79"O	1.59	2005	1320

* Date when the vineyard was established.

2.3.2 Monitoring of *Drosophila suzukii*

Monitoring of *D. suzukii* was carried out using flytraps, made from a 1030 ml plastic pot with a lid containing 27 holes of 0.2 cm in diameter located in the upper third and a red band in the middle. Four traps were placed per vineyard, where two were installed inside the vineyard and the other two at the forest border that surrounds the vineyards. Inside the vineyards, the traps were distributed randomly, while respecting a minimum distance of 15 m from the border; the traps were hung from a shaded branch in the middle third of the plants (Funes *et al.*, 2018; Mazzetto *et al.*, 2015). Traps in the forest border were randomly distributed respecting a minimum distance of 3 m from the vineyard border. Traps were baited with 150 mL of the attractant Droskidrink (one part of red wine, three parts of apple cider vinegar, and 20g L^{-1} sugar) (Grassi *et al.*, 2014). A surfactant (neutral detergent) was added to the attractant to reduce the liquid surface tension and ensure that insects would be trapped (Schlesener *et al.*, 2015).

Weekly the flytraps were monitored by removing the captured insects using a fine mesh sieve and transferring them to 80 mL plastic containers containing 70% alcohol. The attractant in the baits was replaced after the insect removal. The insects captured were directly transferred to the Entomology laboratory of Agroveterinary Sciences College at the Santa Catarina State University (CAV/UDESC), for sorting, identification, and counting of the flies. The identification of drosophilid adults was based on external morphology and analysis of the genitalia, following the specific key for *D. suzukii* (Vlach, 2010). For males, a dark spot on the wings and pairs of sex combs on the tarsus of the forelegs were observed. In females, a double-serrated ovipositor was observed. All specimens were identified using binocular dissecting microscopes (5×–40×) (Eyemag, Carl-Zeiss). The adults of *D. suzukii* were separated by sex and the sex ratio was calculated using the formula: number of females/(number of males + number of females) captured (Silveira-Neto *et al.*, 1976).

2.3.3 Climate variables

Precipitation, maximum, average, minimum temperature, and relative humidity data were provided by the Information Center for Environmental Resources and Hydrometeorology of Santa Catarina (Ciram – Epagri), from meteorological stations located close to the vineyards (Epagri, 2020).

2.3.4 Data analysis

The data from the monitoring period were aggregated into descriptive statistics, showing the weekly values of captured insects. The Wilcoxon non-parametric paired test (Montgomery; Runger, 2013) was used to compare the number of insects in the forest border and inside each vineyard, and to compare the yearly totals of *D. suzukii* for the forest border and inside each vineyard. Multiple regression models were used to describe the total number of insects caught in the forest borders and inside the vineyards, as a function of the climate variables. The selection of explanatory variables in the model was performed using the Stepwise method (Venables; Ripley, 2002). All analyses were performed in the R environment (R Core Team, 2021) considering a 5% significance level.

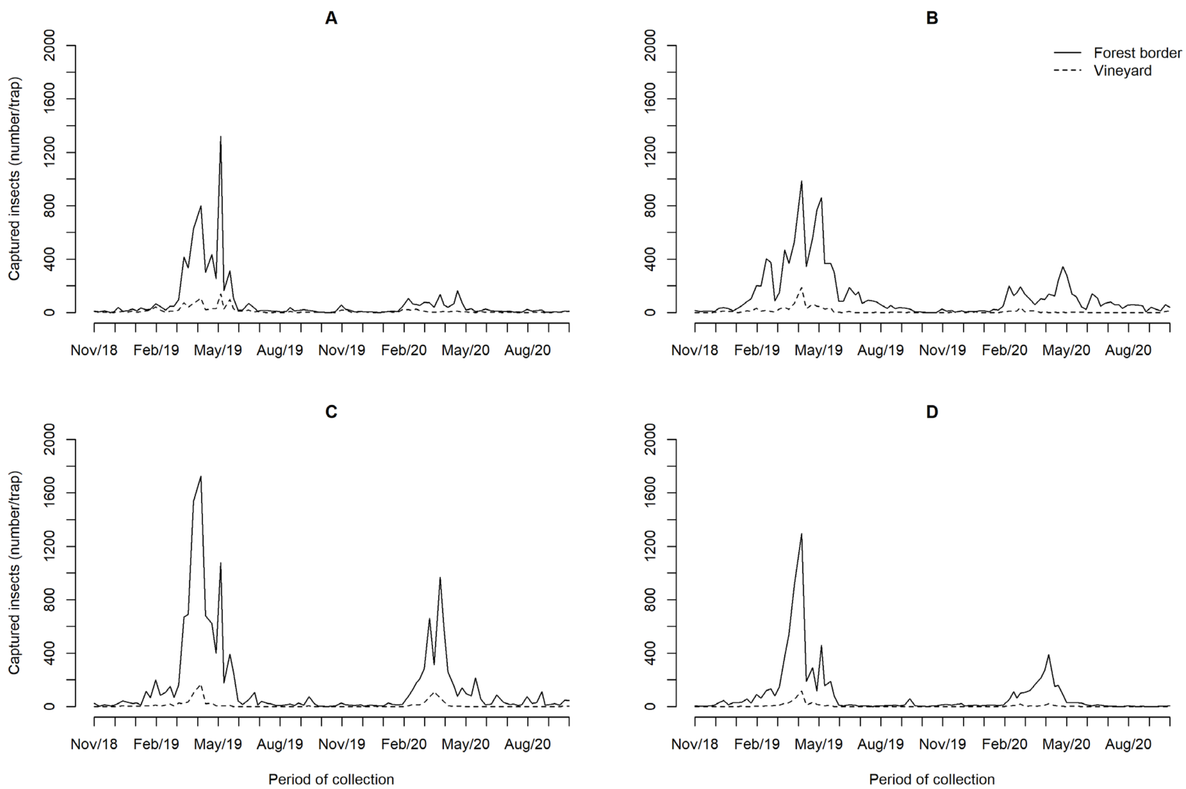
2.4 RESULTS

2.4.1 Population dynamics

During the sampling period, the *D. suzukii* population fluctuated over time at the forest border and inside the vineyard at the four monitored vineyards (Figure 6). Overall, we observe that in some weeks no captures were recorded, but in other weeks more than thousands of flies were caught (SI 1). Despite de variations in the number of the colleting adults, it was possible to confirm that *D. suzukii* is present during all seasons, especially in the forest border, which may be related to its ability to withstand adverse weather conditions throughout all seasons. Considering the periods from November 2018 to October 2019 (year 1) and November 2019 to October 2020 (year 2), it is clear that the catch rate was higher in the first year than in the second year of monitoring. Despite that, the catch peaks showed similar patterns over time, with numbers rapidly increasing around March (end of summer) and returning to low numbers by June/July (winter) (Figure 6).

The number of *D. suzukii* captured in the forest border was consistently higher than in vineyards for all sites and years of monitoring (Figure 6), which may have been favored by the diversity of plant species and the availability of host fruits present in that area. Only during periods of high capture rates at the forest border, flies were also found in substantial numbers in the vineyards. This period also corresponds to the time when the grapes were almost ripe, which might have attracted the flies to disperse inside the vineyards. The number of *D. suzukii* adults captured was different between the vineyards (Figure 6), where vineyard C presented the highest capture rate values of *D. suzukii* adults both at the forest border and inside. On the other hand, vineyards A, B, and D showed similar capture rates to each other and lower than vineyard C.

Figure 6 - Population fluctuation of *Drosophila suzukii* adults inside of Cabernet Sauvignon vineyards (A, B, C, and D) and forest border. São Joaquim, SC, Brazil. Data are average weekly capture per trap.



The sex ratio is an index based on the female proportion of the total number of *D. suzukii* adults captured, where indices above 0.5 indicate more females than males captured. Inside vineyards, the sex ratio indices obtained were above 0.5 between the "*veraison*" stage (when the grapes are changing color) and harvest of Cabernet Sauvignon grape (~ 4 months) (Table 2). This means that there was a greater proportion of female captures. When comparing the sex ratio between the forest border and inside the vineyard, it was consistently and significantly higher within vineyards than in the forest border for all sampled vineyards, except for one case (vineyard A in 2020) (Table 2). This indicates that the female capture rate in the vineyards is not a representation of what happened in the forest border, but is a behavioral preference of females, supporting the hypothesis that females migrate to the vineyards in an attempt to find places for oviposition after the grapes start to change color.

Table 2 - Descriptive statistics comparison for the sex ratio of *Drosophila suzukii* adults captured in the forest border and inside the vineyards. Results of the Wilcoxon paired tests are provided for the phenological stage "*veraison*" until the harvest of Cabernet Sauvignon grape, for each year of monitoring, and between four vineyards in the region of São Joaquim, SC, Brazil

Year	**Site**	**Sex ratio (±SD[1])**		**Median**	**Confidence interval limits**		**p-value[2]**
		Forest border	Vineyard		Lower	Higher	
2019	**A**	0.58±0.21a	0.67±0.23^{NS*}	-0.0964	-0.1600	-0.0396	0.0033
	B	0.47±0.22ab	0.67±0.27	-0.1946	-0.2736	-0.1247	<0.0001
	C	0.54±0.21ab	0.64±0.26	-0.0111	-0.1783	-0.0534	<0.0001
	D	0.46±0.15b	0.60±0.28	-0.1777	-0.2835	-0.0472	0.0161
2020	**A**	0.59±0.25a	0.66±0.23^{NS}	-0.0640	-0.1685	-0.0372	0.1938
	B	0.49±0.18ab	0.69±0.23	-0.2118	-0.3013	-0.1078	<0.0001
	C	0.44±0.16b	0.57±0.31	-0.1645	-0.2762	-0.0507	<0.0001
	D	0.48±0.23ab	0.72±0.27	-0.3074	-0.4287	-0.1784	<0.0001

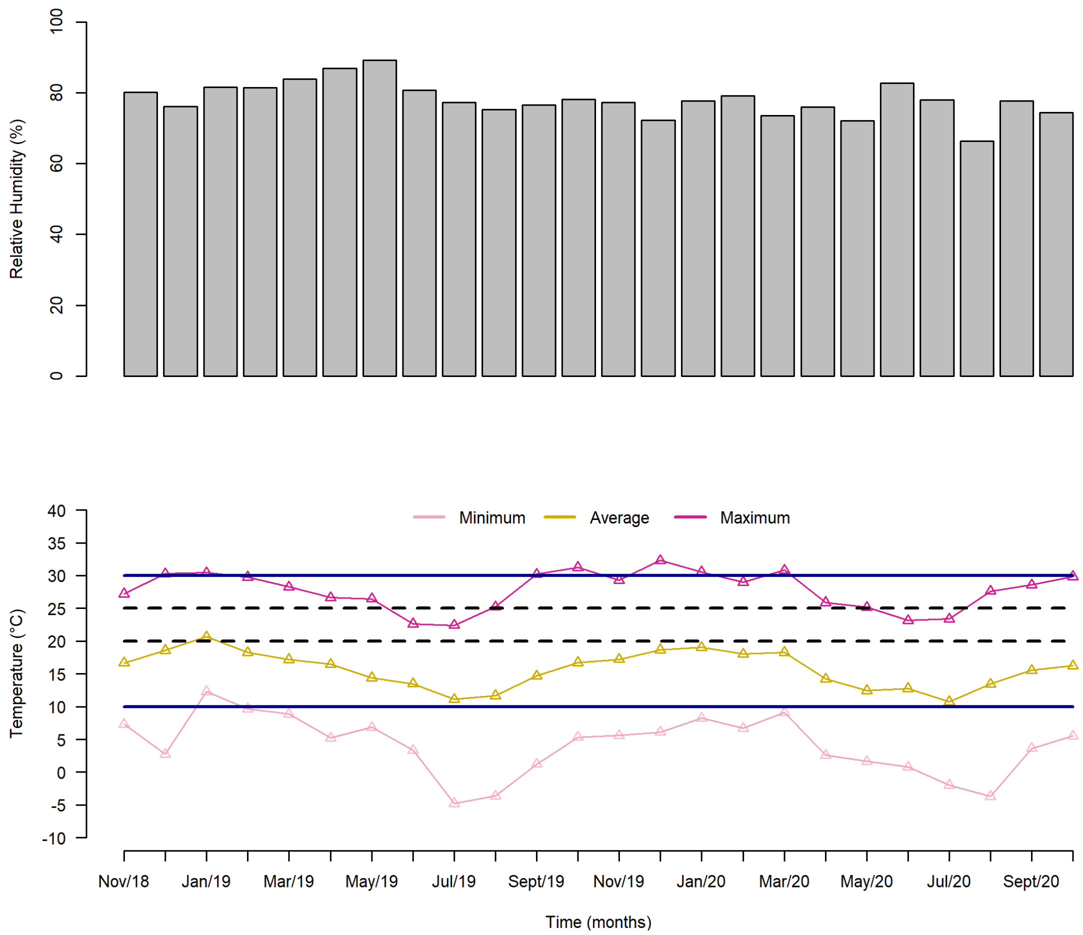
[1] Standard deviation. Sex ratio followed by equal letters, in the year, did not differ according to the Wilcoxon test among the four sites. * NS: not significant. [2] The p-value reports on the Wilcoxon test between the forest border and inside the vineyards.

Comparing the sex ratio between sites within the year, we observed a significant difference in the forest borders between some sites (Table 2). These differences between the sites in the forest border can be related to the natural vegetation composition that brings to each site a specific environment surrounding the vineyards. On the other hand, within the vineyards, no significant differences in the sex ratio of *D. suzukii* adults were observed between sites (Table 2).

2.4.2 Climate variables

The monthly average of relative humidity, and the monthly maximum, average, and minimum temperature are presented for the period from November 2018 to October 2020 in Figure 7.

Figure 7 - Climate variables during the period from November 2018 to October 2020 for São Joaquim, Santa Catarina, Brazil.



Note: The range composed by the dashed lines corresponds to the ideal temperature for *Drosophila suzukii* development, and the range composed by the solid blue line corresponds to the good temperature condition for reproduction (Schlesener *et al.*, 2015).

Multiple linear regression models were used to relate the weekly climatic variables to the *D. suzukii* population fluctuations. The patterns varied across the four vineyard sites monitored (Table 3). Relative humidity was the only climatic variable consistently correlated to the increase in the *D. suzukii* population in all vineyards and forest borders (Table 3). It explained between 1.7 and 18 % of the variation, with the highest contribution observed in site A.

Table 3 - Estimates of multiple linear regression model of the population numbers in the forest border or vineyard (VE) as a function of climatic variables (CV). The presented climatic variables (CV) are those that were retained in the multiple regression model with the stepAIC function from the MASS package in R. This function iteratively adds and removes predictor variables until it reaches a set of predictor variables that produced the model with the lowest AIC value.

Site	VE	CV *	Estimate (±EP)	Explained variability (%)	p-value
A	Forest border	T. maximum	-124.50±49.02	04.61	0.0118
		T. average	122.32±50.82	00.24	0.0170
		Rainfall	-002.65±01.06	04.47	0.0126
		RH	037.35±05.39	11.75	<0.0001
		TR	112.36±27.05	06.57	<0.0001
	Vineyard	T. minimum	-009.73±02.55	01.04	0.0001
		T. average	009.32±02.70	04.56	0.0006
		RH	003.21±00.47	17.96	<0.0001
B	Forest border	T. maximum	019.93±13.36	01.16	0.1374
		T. average	-026.91±14.55	01.22	0.0660
		Rainfall	001.26±00.84	06.28	0.1372
		RH	003.45±01.95	01.74	0.0783
		TR	-030.72±10.50	04.02	0.0038
	Vineyard	T. maximum	002.23±01.00	00.60	0.0265
		T. minimum	-002.21±01.10	01.89	0.0452
		Rainfall	000.20±00.10	06.51	0.0543
		RH	000.74±00.28	03.26	0.0080
		TR	-002.03±01.32	01.12	0.1264
C	Forest border	T. minimum	-091.50±34.76	00.04	0.0093
		T. average	086.90±33.91	00.05	0.0113
		RH	013.77±04.93	05.92	0.0058
		TR	-042.30±23.41	01.92	0.0726
	Vineyard	T. minimum	-005.77±02.62	00.26	0.0288
		T. average	005.73±02.54	00.08	0.0253
		RH	000.98±00.37	04.26	0.0087
D	Forest border	T. minimum	-033.53±18.86	00.80	0.0773
		T. average	032.10±18.34	00.29	0.0820
		Rainfall	001.71±01.06	03.52	0.1100
		RH	006.84±02.80	03.50	0.0155
	Vineyard	RH	000.22±00.07	05.48	0.0027

*T: temperature; RH: relative humidity; TR: thermal range.

The rainfall, thermal amplitude, and maximum and average temperature contributed to both the increase and reduction of *D. suzukii* populations across certain sites (Table 3). These variables explained variations ranging from 0.05% to 6.6%. The minimum temperature was a negative influence (Table 3). The climatic variables rainfall, thermal amplitude, and temperature did not seem a factor in explaining the population numbers in all the vineyards (sites) (Table 3). This result indicates that each vineyard has a specific microclimate that can result in different patterns in the *D. suzukii* population dynamics.

The multiple linear regression indicated how much each climatic variable explained the variability in population numbers. This indicates how much each climate variable influenced the fly's capture in the forest border and inside the vineyard. By adding up the explained variability values for each site, the total variation explained was for a maximum of 28% and a minimum of 5% (Table 3). This indicates that there are still other factors, biotic or abiotic, that are influencing the *D. suzukii* population dynamics in the monitored vineyards.

2.5 DISCUSSION

To gather information about the population dynamics of *D. suzukii* in and around Cabernet Sauvignon vineyards in south Brazil, we monitored their numbers through weekly captures from the forest border and inside the vineyards and correlated this with climate data to analyse which climate variable contributes to population growth. The strongest peaks of *D. suzukii* occurred at the end of summer/beginning of autumn. This period overlaps with the Cabernet Sauvignon grape maturation and susceptible stage for *D. suzukii* damage. Higher capture rates were identified in the forest border than inside the vineyard. Females showed a higher tendency than males to move inside the vineyard during the grape ripening period. Relative humidity was the climate variable that seemed to contribute most to the increase of the *D. suzukii* population in the Cabernet Sauvignon vineyards located in the highland region of Santa Catarina State. This effect of relative humidity can be used to predict the population increase during the pre-harvest of Cabernet Sauvignon grape, as part of an integrated pest management of *D. suzukii*.

2.5.1 Population dynamics

Adults of *D. suzukii* were continuously present during the two years of monitoring, which reflects their ability to survive the different conditions of the seasons. The seasonal survival ability of *D. suzukii* has been reported in several studies, by demonstrating a seasonal phenotypic plasticity of this species (Hamby *et al.*, 2016; Stephens *et al.*, 2015). The phenotypic plasticity of *D. suzukii* is manifested in two morphotypes, winter and summer, which also differ in gene expression and metabolic profile providing different characteristics in cold tolerance (Shearer *et al.*, 2016). The winter morph presents darker body pigmentation and longer wings than summer morphs, has a higher cold-tolerance, and can enter a reproductive diapause when the temperature is below 5 °C; they can withstand dying for temperatures until around -7°C (Funes *et al.*, 2018; Jakobs; Gariepy; Sinclair, 2015; Stephens *et al.*, 2015). This capability may account for the low captures recorded in this study, even during the winter period. Grassi *et al*. (2018) reported that during the diapause period, *D. suzukii* females significantly decrease the levels of mature eggs, which favors the *D. suzukii* population decline during the winter in cherry areas. Although our data does not separate the morphotypes of the captured adults, we did observe the light and dark coloration of the body during the sample identifications and concluded that both morphotypes occur in this region of Brazil.

The capture rates of *D. suzukii* in the forest border were consistently higher than in the vineyards, even reaching a more than 10-fold difference during the peak of the abundance. This observation aligns with findings by Briem *et al*. (2018) who reported a high capture of *D. suzukii* adults in the forest borders compared to inside the vineyards, and numbers of adults during the peak season of more than 10 times higher in the forest border than inside the vineyards located in Germany. Similarly, Groot *et al*. (2021) noted higher capture rates of *D. suzukii* adults in areas surrounding orchards than inside of raspberry orchards in Slovenia.

The abundance of *D. suzukii* can be dependent on the type of habitat (Groot *et al.*, 2021). Forest borders can provide habitat as well as microclimatic conditions favorable for *D. suzukii* reproduction and development (Briem *et al.*, 2018; Weißinger *et al.*, 2019). The availability of wild and native fruits provides both food and breeding resources and favors insect permanence in forest borders (Kenis *et al.*, 2016; Urbaneja-Bernat *et al.*, 2020). Additionally, according to Harris *et al*. (2014) and Mitsui *et al*. (2010), flowers can also provide food resources for *D. suzukii* adults and larvae through nectar or direct feeding on the fleshy parts of flowers. The forest borders around the vineyard where we performed the study are mainly composed of Myrtaceae family species, which can provide availability of fruit, as well as flowers, suitable for *D. suzukii* oviposition and development throughout a longer period of the year. This may explain the higher capture rates occurring in the forest borders than inside the monitored vineyards.

Fewer captures of *D. suzukii* adults in vineyards than in forest borders containing blackberries were observed by Weißinger, Schrieber, *et al*. (2019). The authors state that the greater susceptibility of blackberries compared to grape berries may explain the differences in capture. The abundant presence of *D. suzukii* on the forest border found in this study also suggests that wild fruits are more attractive to flies than grapes, even the females showing a tendency to move inside the vineyards in the final grape ripening period. However, native fruits still serve as hosts, sustaining the *D. suzukii* population until cultivated crops become available in the field, facilitating the seasonal migration of flies into the susceptible crops (Drummond; Ballman; Collins, 2019).

In this study, we identified a single population peak during autumn (between March and June) into the vineyards. It may have occurred due to the combination of grape availability (period of ripening) and favorable climate conditions. *Santoiemma et al*. (2018) observed that the *D. suzukii* population peaks in vineyards located in Verona, Italy, occur in the period that coincides with the grape harvest. A single population peak during the summer in the vineyards (ripening period) was also observed by *Briem et al*. (2018) in Germany. The authors suggest that the grapes are not suitable for hosting the pest in the other year seasons due to the fruit availability and microclimate conditions. This induces an annual migration to the vineyards, specifically during the ripening period, while forest areas seem to be the preferred habitat year-round. Furthermore, Briem *et al.* (2018) report that the *D. suzukii* peak in forest borders or orchards changes depending on the season. In this study, it is possible to observe that although occurring in the same period, the population peaks were different between the observed years. This may be related to the climatic conditions and phenological grape stage, which can change each year, as observed by Groot *et al*. (2021) through monitoring in raspberry orchards in Slovenia. In the second year of our study, we noted a reduction in relative humidity and an increase in temperature during the ripening period compared to the first year.

The vine cultivation system can contribute to the vineyard having an unfavorable microclimate for *D. suzukii*. In the monitored vineyards, the conduction system used is espalier, which provides a greater incidence of luminosity in the vegetative canopy and exposure of bunches to the sun, in addition to providing good aeration and facilitating mechanized operations (Miele; Mandelli, 2012). This conduction system makes the microclimate close to the grape bunches hotter and drier, disfavouring the permanence of *D. suzukii* in the area. Regardless of the management adopted, Diepenbrock, Burrack (2017) observed a higher natural infestation index in blackberry fruits located inside the canopy of plants because of the higher relative humidity indices. The authors state that the microclimate of the plant is what influences the *D. suzukii* infestation and not the availability of fruits.

In this study, we observed sex ratio indexes above 0.5 during the Cabernet Sauvignon ripening period inside the vineyard, which means a proportionally greater *D. suzukii* female capture. However, for the forest border, not all the sites showed sex ratio indexes above 0.5 for the same period. Supporting our findings, Weißinger, Samuel, *et al*. (2019) observed that during grape maturation the sex ratio changed to a greater male capture in the forest border, which is in line with especially the females migrating into the vineyards. Females prefer fruits at an advanced ripening stage since very acidic fruits hinder the development of larvae (Schlesener *et al.*, 2015). The greater female presence inside the vineyards confirms that when the grapes begin the phenological stage of ripening, they become attractive to *D. suzukii* females that are looking for fruits to lay eggs.

By collecting samples of the grapes in the field (vineyard), we did not identify a natural infestation, because no *D. suzukii* adults emerged. This may be related to the fact that the grape is not considered a good or a preferred host for this pest (Ioriatti *et al.*, 2015). However, *D. suzukii* oviposition can still have significant consequences, facilitating pathogen infections, accelerating fruit decay, and contributing to acid rot epidemics in vineyards, thereby impacting the chemical composition and quality of must and wine (Barata; Malfeito-Ferreira; Loureiro, 2012; Ioriatti *et al.*, 2018). Moreover, *D. suzukii* oviposition can lead to negative consequences such as cracking and the attraction of wasps, bees, and birds, during the harvest period (Atallah *et al.*, 2014; Ioriatti *et al.*, 2015). Despite the smaller number of *D. suzukii* adults captured inside the vineyards compared to the forest border, it is crucial to recognize the potential migration of females into vineyards during grape maturation. Therefore, it is recommended that farmers pay attention and establish a monitoring and management system to mitigate losses caused by indirect damage from *D. suzukii.*

2.5.2 Climate variables

The adaptation of an exotic organism to a new habitat is dependent on biotic and abiotic factors, among which climatic conditions can be decisive for the success of population establishment and adaptation (Begon; Townsend, 2021). Several studies indicate that temperature and humidity affect the *D. suzukii* biology and adaptation (Benito; Lopes-da-Silva; Santos, 2016; Eben *et al.*, 2018; Tochen *et al.*, 2014). In this study, we correlated the *D. suzukii* capture rates to the minimum, average, and maximum temperatures, relative humidity, rainfall, and thermal amplitude.

Among all the climatic variables analyzed in this study, relative humidity was the only climatic variable to consistently influence the maintenance of the *D. suzukii* population in all four monitored sites. This corresponds with the findings in other research, that also identified high relative humidity as the best variable to estimate the abundance of *D. suzukii* summer morphotypes and females (Guédot; Avanesyan; Hietala-Henschell, 2018). Tochen *et al*. (2016) observed in the laboratory that relative humidity levels above 20% increase the reproductive potential of *D. suzukii*, and the higher the relative humidity, the greater the intrinsic rate of population growth. The authors also identified a positive correlation between relative humidity and *D. suzukii* captures in blueberry orchards in the state of Oregon, U.S.A.

During the entire monitoring period for this study, the relative humidity ranged from 65 to 90%. These values can be considered favorable for the establishment of the *D. suzukii* population in vineyards located in the highland area of Santa Catarina state. Tochen *et al*. (2014) stated that cultural practices can reduce the relative humidity in the field, resulting in a reduction in the *D. suzukii* population. This statement reinforces the previously pointed out hypothesis that the vine training system Espalier used may be contributing to the low capture inside the vineyards. Although relative humidity was shown to positively influence the *D. suzukii* population in all monitored sites, it is not as well investigated as temperature effects (Evans; Toews; Sial, 2018; Tochen *et al.*, 2014; Wiman *et al.*, 2014).

Maximum average temperatures (>30°C) are the main ecological factor to limit the spread of *D. suzukii* in Brazil (Benito; Lopes-da-Silva; Santos, 2016). In the laboratory, Tochen *et al*. (2014) observed that at a temperature of 22 °C, the intrinsic rate of population increase of *D. suzukii* was higher (0.22) when compared to other temperatures in a range from 14 to 28 °C. Evans *et al*. (2018), also in the laboratory, observed that heat stress (>30 °C) can reduce the fecundity and fertility of *D. suzukii* females. In contrast with our findings, other studies did find that fluctuations in the *D. suzukii* capture rates were correlated mostly to temperature in orchards of several fruit trees in another region of Brazil, as well as in Greece (Papanastasiou *et al.*, 2020; Wollmann *et al.*, 2019). In our study, temperature did explain a significant, but mostly small part of the variation in capture rate. During almost the entire period of this study, the average temperature oscillated within the *D. suzukii* developmental temperature limits (10 – 30°C) (Schlesener *et al.*, 2015). This may have favored reproduction even in the coldest months (June to August).

In the laboratory, Eben *et al*. (2018) carried out studies and correlated temperature and humidity to understand whether both can either act together or act independently in the survival of *D. suzukii* adults. The authors observed an age-dependent increase in mortality of *D. suzukii* adults when exposed to extreme heat and low humidity. Guédot *et al*. (2018) also identified a correlation between temperature and humidity in the *D. suzukii* population and claimed that this combination was the best predictor. In our study, the conditional effects model automatically selected the climate variable that was most correlated with the capture rate of *D. suzukii* adults. However, it does not mean that there was no temperature effect per se. It just shows that, because of the correlation between temperature and humidity, when the humidity is included in the model, the temperature effect does not increase the model quality significantly.

In addition to temperature and relative humidity, rainfall and temperature range were found to correlate with *D. suzukii* capture rates, but not always in the same way. Each monitored vineyard exhibited a unique microclimate, which can be observed from the different influences that the climatic variables had on the *D. suzukii* population for each site. Corroborating these results, Wiman *et al*. (2014) observed in four different locations that the micro-ecosystem had the greatest influence on the behavior of the *D. suzukii* population. The authors state that this may have implications for *D. suzukii* management since it implies that the population structure and behavior of this pest may show large differences between agricultural ecosystems.

2.6 CONCLUSION

Through studying the population dynamics, we increase our understanding of *D. suzukii*'s behavior in the field and identify the climatic factors that influence the annual developments in population increase. In this study, the *D. suzukii* adult presence in the forest border and inside the vineyards showed substantial differences. The population started to increase when the temperature increased, but it remained favorable to *D. suzukii* development throughout the monitored period. Relative humidity was also favorable throughout the monitored period, but it seemed to explain a larger part of the variation in trap captures across the monitored sites.

Although native forest borders have shown the highest *D. suzukii* population rates, we do not recommend removing these ecosystems, as they can host beneficial insects as well. We recommend that studies to manage the forest border be developed focusing on keeping the *D. suzukii* adults inside. It could be done by providing more preferred fruits or sentinel plants in the forest border and during the period of grape ripening using mass trapping or bait toxic to prevent them from migrating to inside of the vineyards. We suggest the management of forest borders based on our findings and in previous studies developed in other vineyards where it was possible to see that they do not seem to like grapes and the infestations are smaller in the vineyards. Also, removing the forest border could potentially make the vineyards relatively more attractive for *D. suzukii* adults due to a lack of alternative breeding sites.

The data obtained in this study provide information for the *D. suzukii* management in vineyards located in the highland region of Santa Catarina state, Brazil. Indeed, a significant risk of *D. suzukii* infestations in vineyards exists, but only during relatively short periods of the year and specifically during the grape ripening period. Aiming for sustainable *D. suzukii* management, we suggest that more research using toxic baits, mass capture, and natural insecticides be carried out, focusing on the “*veraison*” stage, to offer management possibilities to the farmers.

Supplementary Information

Supplementary Information 1. Descriptive statistics on the total number of *Drosophila suzukii* adults collected in forest borders and vineyards for the four monitored locations (A, B, C, and D). São Joaquim, SC, Brazil.

Period	Site	Forest border				
		Minimum	Maximum	Average	Median	SD[1]
2018/19	**A**	0	1639	119.07	21.0	252.13
	B	0	1046	180.30	73.0	240.65
	C	0	2927	197.99	29.5	436.82
	D	0	1696	113.94	16.0	248.78
2019/20	**A**	0	0247	28.46	11.5	39.84
	B	0	0452	73.66	45.0	83.00
	C	0	1290	102.17	24.5	194.10
	D	0	0474	45.35	10.0	80.34
		Vineyard				
2018/19	**A**	0	150	18.20	5.5	31.00
	B	0	226	15.13	3.0	30.99
	C	0	196	10.25	1.5	28.18
	D	0	147	6.95	0.0	19.34
2019/20	**A**	0	041	5.87	4.0	7.51
	B	0	040	3.71	1.0	6.32
	C	0	166	7.72	0.0	22.25
	D	0	032	2.56	1.0	5.07

[1] Standard deviation.

3 *Drosophila suzukii* POPULATION DYNAMICS IN VINEYARDS AND WINE CULTIVARS SUSCEPTIBILITY*

3.1 ABSTRACT

Grapes are not considered a good host for the spotted-wing drosophila, *Drosophila suzukii*. However, its damage results in qualitative and quantitative losses to the wine grapes berries. Knowing the *D. suzukii* population dynamics in vineyards and surrounding areas, as well as the wine cultivars' susceptibility, can help winegrowers carry out the management. The objective of this study was to evaluate the *D. suzukii* population dynamics in the field, on the forest border present around the vineyard and in its interior, and to evaluate the susceptibility of the main wine cultivars for São Joaquim municipality to the attack of *D. suzukii* in the laboratory. In the vineyard, *D. suzukii* was monitored from November 2018 to May 2021 with weekly inspections. Plastic pot traps with a red stripe baited with the Droskidrink attractant were used and distributed among the vines and around the vineyard. Monitoring data were correlated with regional climate variables. In the laboratory, through non-choice and free-choice tests, the susceptibility to *D. suzukii* damage was evaluated in Chardonnay, Sauvignon Blanc, Sangiovese, Cabernet Franc, Cabernet Sauvignon and Merlot grapes. Intact and artificially injured grapes were offered to adults in plastic-screened cages for 24 hours. Afterward, the number of punctures, eggs, emerged adults, and the period of egg-adult development were evaluated. Through monitoring, it was found that the presence of *D. suzukii* adults was higher in the forest border (maximum weekly catch: 762) in the surroundings compared to inside of the vineyard (maximum weekly catch: 128). The precipitation reduced the population of *D. suzukii* inside the vineyard, while the average and maximum temperatures promoted an increase in the population. In free choice tests, the cultivar Sangiovese was susceptible to the attack and development of *D. suzukii*. The cultivar Cabernet Sauvignon showed reduced egg-adult development time compared to the other tested cultivars in a no-choice test.

Keywords: spotted-wing drosophila, monitoring, grape wine, integrated pest management.

3.2 INTRODUCTION

The Brazilian cultivation of wine grapes has stood out recently due to the domestic consumption and export of wines and sparkling wines, resulting from the unique "*terroir*" of the regions where the vines are grown (Mello; Machado, 2020). The main Brazilian region for cultivating wine grapes has a temperate climate, traditional in the South and the Southeast altitude regions (São Paulo and Minas Gerais). This temperate climate cultivation region is characterized by an annual cycle, followed by a dormancy period induced by low winter temperatures (Camargo; Tonietto; Hoffmann, 2011). São Joaquim is a municipality located in the Santa Catarina highland region (Camargo; Tonietto; Hoffmann, 2011), a center of one of the country's youngest grape wine regions. In the year 2021, Santa Catarina highland region, which is considered the coldest and highest altitude in the south of Brazil, obtained the seal of "Geographical Indication of Santa Catarina Altitude Wines", bringing prominence at a national level and increasing value to the wines produced in this region.

The sanitation of the vines as well as the quality of the grape bunches is important to ensure high production and the wine's quality. The invasive pest *Drosophila suzukii* species (Matsumura, 1931) (Diptera: Drosophilidae) has recently been detected for causing damage to the wine grape, compromising the production and the wine's quality produced in the Santa Catarina highland region. Originally from Japan, *D. suzukii* is a polyphagous quarantine pest, popularly known as the Spotted Wing Drosophila – SWD (Berry, 2012), and is in constant global expansion. Since 2008, it has caused significant economic damage to small fruits and fruits with soft skin, in European and North American countries (Calabria *et al*., 2012; Goodhue *et al*., 2011; Hauser, Gaimari, Damus, 2009; Schlesener *et al*., 2015). In Brazil, *D. suzukii* was identified initially occurring in the Southern and Southeast states (Deprá *et al.*, 2014; Vilela; Mori, 2014), causing damage in strawberry, blueberry, peach, plum and feijoa (Geisler *et al.*, 2015; Santos, 2014; Souza *et al.*, 2017).

Male adult *D. suzukii* are small (3 mm), being identified mainly by the characteristic black spot on their wings. The females do not present spots on the wings and measure 4 mm in length. They present a serrated ovipositor, which differentiates them from the other *Drosophila* species (Schlesener *et al.*, 2015). *Drosophila suzukii* is a polyphagous pest that has a wide range of hosts (Walsh *et al.*, 2011). Its serrated ovipositor enables the oviposition just underneath the skin of fresh soft fruits that still are in the pre-harvest period (Hauser, 2011; Mitsui; Beppu; Kimura, 2010).

In the grapes, the damage results both from the oviposition puncture and from the larvae that consume the pulp (Ioriatti *et al*., 2015, Kanzawa, 1939). Recent studies have shown that in white table grapes ('Thompson'), *D. suzukii* has reduced oviposition, a prolonged biological cycle, and lower survival, compared to the larvae that develop in other fruits, such as raspberries and cherries (Lee *et al.*, 2011). Although both table and wine grapes are not considered preferred hosts, Bellamy *et al*. (2013) and Wang, Kaçar, Daane (2019) reported that *D. suzukii* can complete its life cycle in both table and wine grapes.

In wine grapes, *D. suzukii* damage present negative impacts such as berry crack, the penetration of pathogenic agents, and the attraction of other animals during the harvest period due to juice extravasation, besides the presence of larvae in the must during processing (Atallah *et al.*, 2014; Ioriatti *et al.*, 2015). Also, studies by Barata *et al*. (2012) and Ioriatti *et al*. (2018) showed that *D. suzukii* has the ability to disseminate phytopathogenic agents that cause sour rot epidemics or acid rot in vineyards, which depreciate the chemical composition and the quality of the must and wine.

The phenological cycle duration can influence the susceptibility of the wine grapes to *D. suzukii* damage. Late-cycle cultivars can be at greater risk of *D. suzukii* oviposition since the populations at the end of the summer are larger than at the beginning (Shrader; Burrack; Pfeiffer, 2019). The subtle physicochemical characteristics of each grape cultivar, also contribute to the susceptibility to *D. suzukii* attack. Recent studies in the laboratory reveal that the skin hardness and the soluble solids content influence the *D. suzukii* oviposition rates (Baser *et al.*, 2018; Shrader; Burrack; Pfeiffer, 2019; Tonina *et al.*, 2020).

The climatic conditions characteristic of Brazil's temperate climate region also favor the development of *D. suzukii* (Benito; Lopes-da-Silva; Santos, 2016). It was already identified in the vineyards of São Joaquim, Santa Catarina, Brazil (Padilha *et al.*, 2016)(Benito; Lopes-da-Silva; Santos, 2016). Because it is a recent pest in the Southern region, as in Brazil itself, there is a lack of technical information that assists the farmer in the control of *D. suzukii*. Some preliminary studies were performed on the susceptibility of grape cultivars in Rio Grande do Sul (Andreazza *et al.*, 2016) and food attractions for the capture of *D. suzukii* in commercial vineyards in Santa Catarina, Brazil (Padilha *et al*., 2016). Due to the importance, there is the need to perform new research, which together, can assist in the organization of an efficient management plan against *D. suzukii*.

In the temperate climate vineyards of Santa Catarina highland area in the south of Brazil, the *D. suzukii* population dynamics and the susceptibility of the main cultivated wine grapes are unknown. Understanding the role of the non-agricultural habitats in the *D. suzukii* population dynamics, as well as the differences in the preference and susceptibility of the wine grapes, can assist in the optimization of pest management techniques. Thus, the aim of this study was to evaluate the *D. suzukii* population dynamics in the native forest border present in the vineyard surroundings and inside vineyards, as well as to evaluate the susceptibility of the main wine cultivars for São Joaquim municipality to the *D. suzukii* attack.

3.3 MATERIAL AND METHODS

3.3.1 *Drosophila suzukii* monitoring

The study was carried out from November 2018 to May 2021 in a 3.78 ha vineyard (germplasm bank *in vivo*) of the São Joaquim Experimental Station (EESJ), one of the research units of the Santa Catarina Agricultural Research and Rural Extension Company (EESJ – EPAGRI). The vineyard is located in São Joaquim, Santa Catarina, Brazil, at an altitude of 1440 m (28°14'12.81"S 50° 4'20.84"O). The vineyard is composed of 54 wine grape cultivars (*Vitis vinifera*) with approximately 20 years of implantation, conducted in an espalier system.

Part of the vineyard surroundings corresponds to a forest composed of native forest, characteristic of the Montana Mixed Ombrophila Forest (FOM). The north and south sides are mixed with eucalyptus (*Eucalyptus* spp.) and Cypress (*Cupressus* spp.) used as windbreakers (Figure 8). The east and west sides of the vineyards are bordered by fruit orchards with apple, pear, and feijoa (Figure 8), which are part of the agricultural activity of São Joaquim, Santa Catarina, Brazil.

The monitoring of *D. suzukii* was performed through the installation of eight flytraps, four installed inside of the vineyard and another four in the forest border. The "flytraps" were composed of a plastic pot of approximately 1000 ml with a lid, with a red band of 17.5 cm around the pot. In the middle third of the trap, three groups of 9 holes of 0.2 cm in diameter were located, totaling 27 entry points for the insects. During the monitoring, the traps were filled with 150 mL of Droskidrink as an attractant (one part of red wine, three parts of apple vinegar, and 20g of sugar L^{-1}) (Grassi *et al.*, 2014). Two droplets (approximately 20µl) of neutral detergent were added to the attractant solution to decrease the liquid surface tension and ensure the trapping of the captured insects (Schlesener *et al.*, 2015).

Figure 8 - Satellite image locating the experimental vineyard at the São Joaquim Experimental Station of the Santa Catarina Agricultural Research and Rural Extension Company, and the surrounding plant composition.

Source: Google Earth, 2023.

In the vineyard, the traps containing the attractant were arranged in shaded sites in the middle third of the plants (approximately 1.6 m high) and randomly distributed while respecting a minimum of 15 m away from the border (Funes *et al.*, 2018; Mazzetto *et al.*, 2015). The traps installed outside the vineyard were positioned in the north and south points (inside the forest), respecting a minimum distance of 15 m from the vineyard border. Weekly, the traps were inspected by collecting the captured insects with the aid of a fine mesh sieve. The attractant was replaced during the collection of the insects.

The captured insects were placed in 80 mL plastic containers containing 70% alcohol and properly labeled. Then, this material was forwarded to the Entomology laboratory of the Center for Agroveterinary Sciences at Santa Catarina State University (CAV/UDESC) for the screening and counting of the captured *D. suzukii* male and female adults.

The identification of the *D. suzukii* adults was performed based on the external morphology and genitalia analysis, following the specific key for *D. suzukii* (Vlach, 2010). For the males, the dark spot on the wings and the pairs of sex combs on the front legs were used for identification. In females, the serrated ovipositor was used. These observations were performed with the aid of a stereoscopic binocular magnifier with 5x increase (Eyemag, Carl-Zeiss). After the screening and identification, *D. suzukii* adults were separated by sex and counted for each trap.

3.3.2 Susceptibility of wine grape cultivars to *Drosophila suzukii* damage

To compare the susceptibility of different cultivars, bioassays were conducted in the Entomology laboratory of the Center of Agroveterinary Sciences (CAV) at Santa Catarina State University (UDESC), under controlled laboratory conditions. The assays were conducted at a temperature of 25 ± 2 ℃, RH 65 ± 10%, and 12 hours of photophase, following the methodology described by Ioriatti *et al*., 2015. The insects used in the bioassays were obtained from the breeding of *D. suzukii* kept in the laboratory under the same conditions as the test was carried out. The population of *Drosophila suzukii* was maintained in plastic-screened cages (28 x 28 x 28 cm). The adults were fed with artificial diet based on cornmeal (80 g), yeast (40 g), sugar (100 g), agar (8 g), propionic acid (3 ml), Nipagin (8 ml, in a 10% alcohol dilution) and 1 L of distilled water (Matsubayashi, Matsuda, Tomimura, Shibata, & Tobari, 1992) according to Schlesener *et al*. (2018) recommendation.

In the field, during the phenological stage, bunches of the tested wine grapes were bagged with 'TNT' tissue bags (non-woven fabric) in order to prevent *D. suzukii* natural infestation. During the harvest, a sample of bunches without bagging was collected for the observation of natural infestation. Throughout the grape maturation period, no insecticides were applied in the vineyard.

To test the susceptibility of the wine grape cultivars to *D. suzukii* damage, we performed three bioassays in the laboratory: i) non-choice with grapes without artificial damage; ii) non-choice with grapes with artificial damage; and iii) free-choice with grapes without artificial damage. In all the bioassays, the insects were deprived of an artificial diet for a period of 12 hours before the release.

Bioassay non-choice – The grape cultivars selected for the bioassays non-choice were Chardonnay and Sauvignon Blanc (white cultivars), and Sangiovese, Cabernet Franc, and Cabernet Sauvignon (red cultivars). The experimental units of the non-choice tests consisted of transparent plastic pots (500 mL), containing a hollow bottom and closed with voile tissue and cap size in a plastic cover (11 cm ø) lined with filter paper.

For the non-choice bioassays using grapes without damage, ten intact grapes of the respective varieties were offered in each cage containing four *D. suzukii* females and one male. The bioassay was performed in a completely randomized design with respect to cultivars assigned to cages, with ten repetitions per treatment. For the non-choice bioassay using grapes with artificial damage, six grapes were used with a cut of approximately 1cm, made with a scalpel sterilized in 99% ethanol. The bioassay was performed in a completely randomized design, with six repetitions per treatment.

Bioassays free-choice – eight *D. suzukii* females and two males were provided with six grapes of each cultivar, in experimental units that consisted of a plastic cage (28 x 28 x 28 cm). The grape cultivars tested were Chardonnay, Sauvignon Blanc, Cabernet Franc, and Sangiovese. The bioassay was performed in a randomized block design with six repetitions.

In all the bioassays, *D. suzukii* adults were removed after 24 h of exposure, and the punctures and eggs deposited within the berries were counted with the aid of a stereoscopic binocular magnifier with 5x magnification (Eyemag, Carl-Zeiss). The grapes containing *D. suzukii* eggs were stored in translucent plastic containers of 7 cm x 5 cm (diameter x height) and sealed with a micro-perforated cover to allow gas exchange. They were observed daily for 25 days to record adult emergence. We evaluated the embryonic viability, the period of egg-adult development time, and the sex ratio of the offspring. The sex ratio was calculated using the following formula: number of females/(number of males + number of females) emerged (Silveira-Neto *et al*., 1976).

Concomitant to the bioassays, the physicochemical characteristics of the wine grapes cultivars were evaluated. For each cultivar, 20 grapes were evaluated for the soluble solids content (°Brix), total titratable acidity, penetration resistance, and color patterns. The soluble solids content (°Brix) was measured with the aid of a bench portable digital refractometer. The total titratable acidity was obtained by the titration of a grape juice sample, diluted in distilled water, and titrated with NaOH 0.1 N solution, with the aid of an automatic titrator. The penetration resistance was determined in newton (N), with the aid of a digital penetrometer, with a tip of 2 mm and penetration of 10 mm, in the equatorial zone of the fruit. The skin background color was determined by an electronic colorimeter, through the realization of readings in the equatorial region of the fruits and expressed in terms of *hue* angle (h^o) (0° = red; 90° = yellow; 180° = green and 270° = blue) and luminosity (L) that refers to the scale that varies from black (0) to white (100).

3.3.3 Climate Variables

The climatic data were collected daily by the meteorological station located in EESJ – EPAGRI, close to the vineyard, compiled and made available by the climate monitoring system of the Environmental Resources and Hydrometeorology Information Center of Santa Catarina (CIRAM) from EPAGRI (Epagri, 2020). The climate variables observed were: maximum, average, and minimum temperature daily; total precipitation monthly; and average relative humidity monthly.

3.3.4 Data analysis

The data obtained during the monitoring period were aggregated in descriptive statistics, presenting the weekly values of insects captured. For the comparison of the insect number in the forest border and the vineyard, and for comparison among the locations in each year (native forest edges and interior of the vineyard), we used the Wilcoxon nonparametric paired test. For comparison among the seasons, we used the unpaired test (Montgomery; Runger, 2013). The multiple regression model was used to relate the climate variables to the total number of insects in the forest border and inside the vineyards. The selection of the explanatory variables in the model was performed by the Stepwise method based on the value of the Akaike information criterion (Venables; Ripley, 2002).

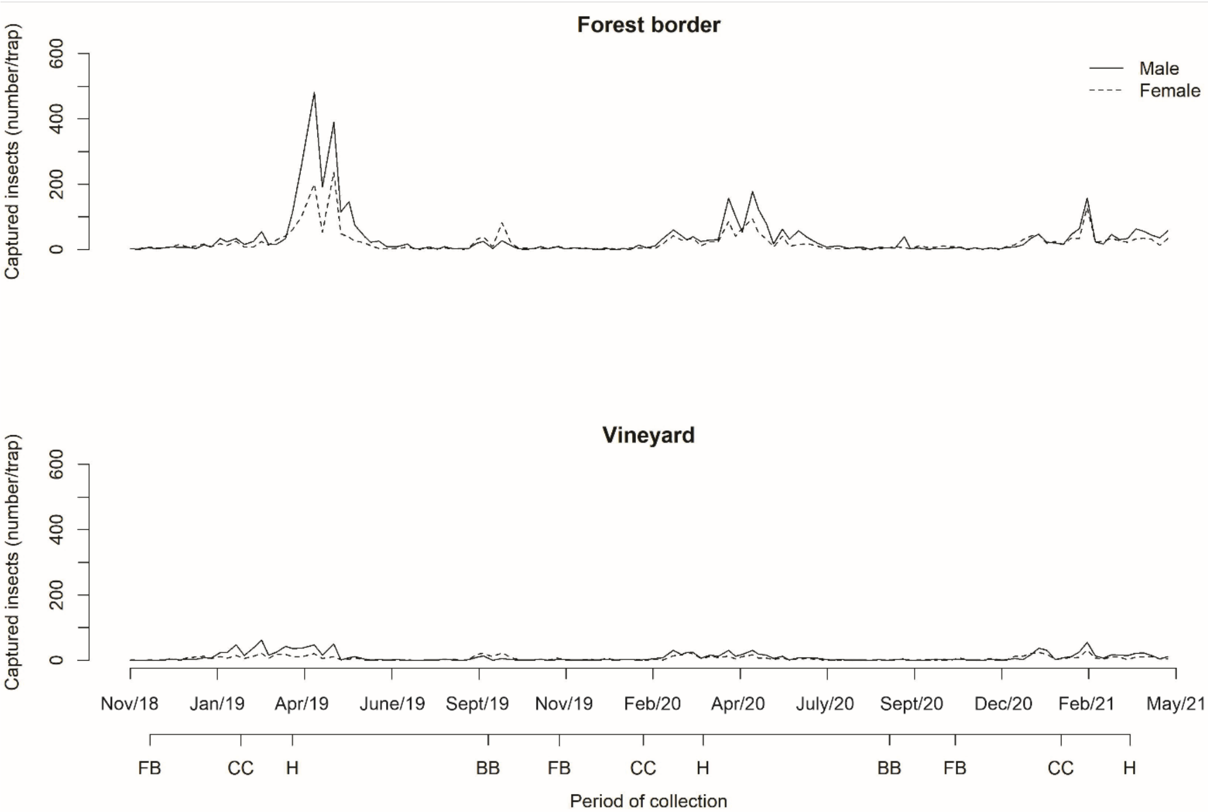
An ANOVA model was used to analyze the cultivars' susceptibility to *D. suzukii* attack data. The assumptions of normality and variance homogeneity were verified, and when one of the assumptions was not met, we used the transformation with Box-Cox optimal lambda (Venables; Ripley, 2002). All the analyses were performed in the R environment (R Core Team, 2021), considering the 5% level of significance.

3.4 RESULTS

3.4.1 Monitoring of *Drosophila suzukii*

During the monitoring period of *D. suzukii* in the EESJ-EPAGRI vineyard, the population peaks in the years 2019 and 2020 occurred between April and May, with a smaller peak in September. In 2021, the population peak occurred in February (Figure 9).

Figure 9 - *Drosophila suzukii* population fluctuation inside of the vineyard and in the surrounding forest border. São Joaquim, SC, November 2018 to May 2021. Data are weekly catch averages.



FB: full bloom; CC: color change (50%); H: harvest; and BB: budburst.

Throughout the monitoring period, the population of *D. suzukii* in the forest border was larger than the one observed inside the vineyard. Only in February 2019, the population of *D. suzukii* inside the vineyard was larger than in the forest border (Figure 9).

The number of caught males was superior to the females during the largest population peaks observed in each year of monitoring for the forest border and vineyard (Figure 9). However, during the population peaks that occurred in September 2019 and between September and December 2020, the number of females captured was higher than the number of males in the forest border (Figure 9). Inside the vineyard, only during the population peak in September 2019, the number of females captured exceeded the number of males, which was similar to what was observed in the forest border (Figure 9). The largest population peaks of *D. suzukii* inside the vineyard corresponded to the period of the wine grapes fruiting (Figure 9).

The data obtained through the weekly collections were aggregated for the grape season, which corresponds to the period of July to June, for each of the three grape seasons of monitoring (Table 4). Only in the 2018/19 season, *D. suzukii* adults were captured every week in the forest border in the surrounding of the vineyards; this was where the highest values of *D. suzukii* adult's captures were recorded (Table 4). Inside the vineyard, the highest indices of captures occurred in the 2020/21 harvest season (Table 4). In both monitored locations, forest border and inside the vineyard, the 2019/20 season was the one in which the lowest indices of *D. suzukii* adult captures were recorded (Table 4).

Table 4 - Descriptive statistics related to the total number of *Drosophila suzukii* adults were collected, weekly, in forest border of the vineyard surroundings and inside of the vineyard, during three monitored harvests from November 2018 to May 2021. São Joaquim, Santa Catarina, Brazil.

Season	Forest border				
	Minimum	Maximum	Average	Median	SD[1]
2018/19	3	762	102.50	37.0a	167.44
2019/20	0	320	46.04	16.5a	63.17
2020/21	0	402	48.27	28.0a	60.69
All 3 years	0	762	59.05	24.0	96.53
	Inside the vineyard				
2018/19	0	103	24.26	15.5b	24.80
2019/20	0	64	13.66	06.0b	15.86
2020/21	0	128	17.94	10.0b	22.63
All 3 years	0	128	17.81	9.0	21.37

[1] Standard deviation. Medians followed by equal letters, in the season, do not differ according to the Wilcoxon test.

The sex ratio consists of the proportion of females of the total number of *D. suzukii* adults. We observed significant differences among the sex ratio obtained in the forest border and inside of the vineyard only for the 2020/21 season, where the sex ratio in the forest border was higher than in the vineyard (Table 5). When comparing the sex ratio among the seasons within the collection sites, we observed significant differences only in the forest border, where the sex ratio during the 2020/21 season was higher and differed from the 2018/19 season (Table 5).

Table 5 - Descriptive statistics and Wilcoxon paired test results for the *Drosophila suzukii* adult sex ratio captured in the forest border of the vineyard surroundings and inside of the vineyard from November 2018 to May 2021. São Joaquim, Santa Catarina, Brazil.

Season	**Sex ratio (±SD[1])**		**Median**	**Confidence interval limits**		**p-value[2]**
	Forest	Vineyard		Inferior	Superior	
2018/19	0.41±0.19b	0.40±0.25[NS]	0.0019	-0.0583	0.0603	0.9618
2019/20	0.46±0.22ab	0.46±0.25	-0.0178	-0.0707	0.0382	0.5155
2020/21	0.49±0.20a	0.44±0.27	0.0548	0.0020	0.1019	0.0410
All 3 season	0.46±0.21	0.44±0.26	0.0172	-0.0143	0.0484	0.2973

[1] Standard deviation. Sex ratio followed by equal letters do not differ according to the Wilcoxon test. [NS] not significant. [2] The p-value reports on the Wilcoxon test between the forest border and inside the vineyards.

The positive and negative values of the multiple linear regression model estimates indicate how each weekly climate variable correlated with the weekly *D. suzukii* population numbers in the forest border and inside the vineyard. The explained variability shows, in percentage, how much of the variability observed in the *D. suzukii* population can be attributed to each variable analyzed considering the sequential model. The explained variability in total was approximately 23% both for the forest border and inside of the vineyard (Table 6). This shows that there is still approximately 77% of the variability that is explained by other factors that influence and affect the *D. suzukii* population. The strongest positive factor for population numbers was temperature (maximum temperature in the forest border; average temperature in the vineyard). Rainfall was the climate variable that negatively influenced the *D. suzukii* population both in the forest border and inside the vineyard (Table 6).

Regarding the climate variables, it was observed that in the year of 2019 in June and August, the rainfall values were below 50 mm, and May presented rainfall values close to 350 mm (Figure 10). In the year 2020, March and July presented the lowest and highest value for precipitation, respectively. In the year 2021, January presented precipitation close to 300 mm and April presented precipitation below 50 mm. The relative humidity oscillated during the period analyzed between 65 and 95%, presenting maximum values in April 2019 and minimum in August 2020 (Figure 10).

Table 6 - Estimates of the multiple linear regression model of the variables evaluated according to climate variables for São Joaquim Experimental Station vineyard of the Agricultural Research Company and Rural Extension of Santa Catarina. São Joaquim, Santa Catarina, Brazil. November of 2018 to May of 2021. The presented climatic variables are those that were retained in the multiple regression model with the stepAIC function from the MASS package in R. This function iteratively adds and removes predictor variables until it reaches a set of predictor variables that produced the model with the lowest AIC value.

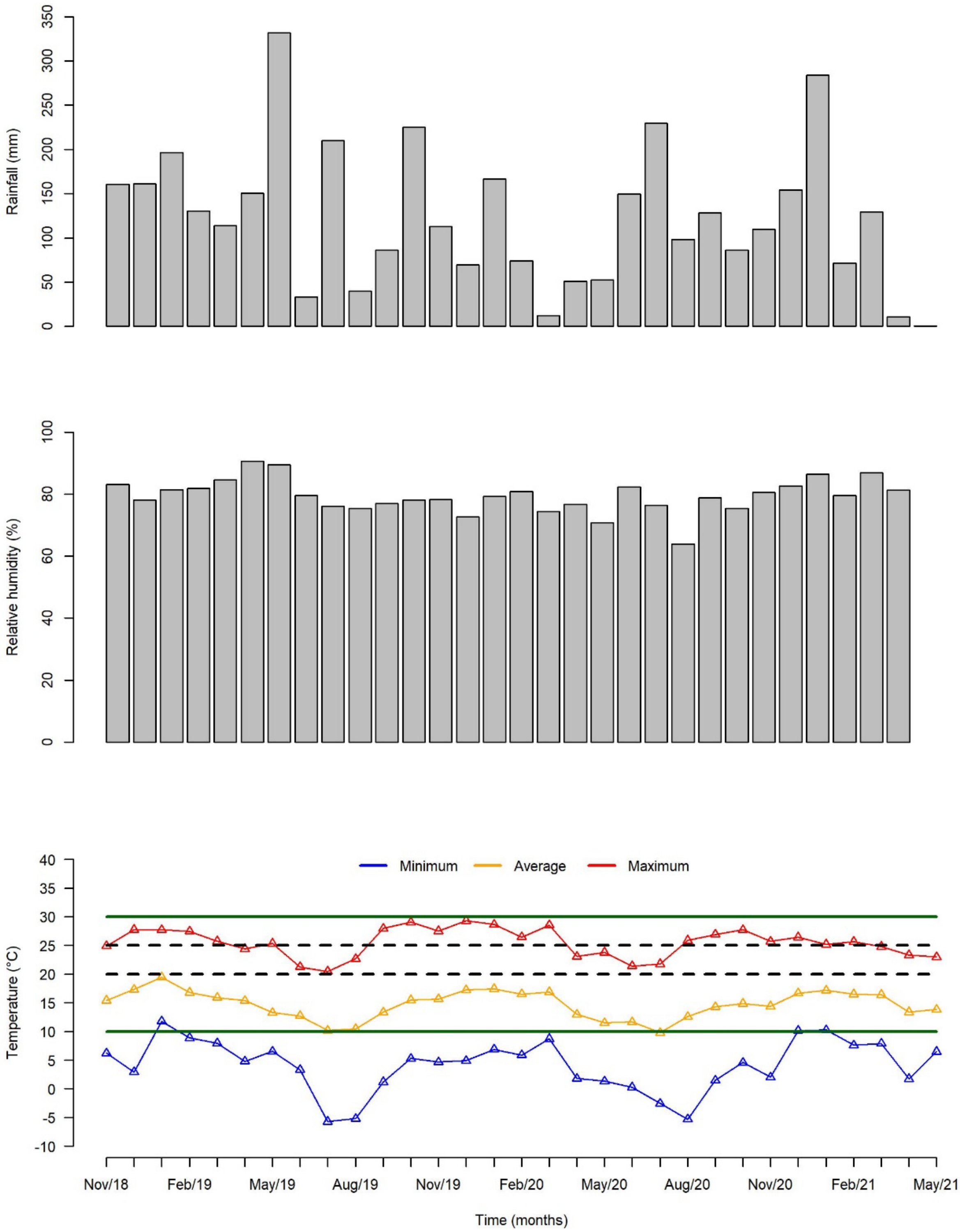
Variables evaluated	Climate variables	Estimates (±EP)	Explained variability (%)	p-value
Forest Border	Maximum temperature	732.44±75.96	11.61	2.5166×10^{-19}
	Rainfall	-000.04±00.01	12.46	1.7864×10^{-11}
Vineyard	Average temperature	192.08±19.75	15.73	1.3701×10^{-19}
	Rainfall	-000.01±00.00	02.43	2.2748×10^{-08}
	Relative humidity	-034.54±06.90	06.34	9.5972×10^{-07}

In the EESJ-EPAGRI, throughout the monitoring period, the minimum temperature presented negative values in July and August for the years 2019 and 2020 (Figure 10). The maximum temperature oscillated between 20 and 30 ℃, and in June, July, and August of 2019, April, May, June, and July 2020, and April and May 2021, remained within the range of 20 to 25 ℃ which corresponds to temperature for the optimal *D. suzukii* development (Figure 10). The average temperature varied, approximately, between 10 and 20 ℃ remaining within the temperature limits (10 to 30 ℃) for the development of *D. suzukii*.

3.4.2 Wine cultivars susceptibility

In the bioassays, all tested cultivars showed susceptibility to infestation, because all presented punctures and *D. suzukii* eggs. Grapes of all cultivars also provided sufficient nutritional conditions for larval development, resulting in the emergence of adults. However, there were differences among the cultivars for the variables analyzed (Table 7).

Figure 10 - Monthly climate variables during the period from November 2018 to May 2021. São Joaquim, Santa Catarina, Brazil.



Observation: The range composed by the dashed lines corresponds to the ideal temperature for *Drosophila suzukii* development. The range composed by the solid green line corresponds to the temperature limits for *Drosophila suzukii* development.

Among the variables analyzed in the non-choice bioassay with grapes without damage, there was a significant difference only for the sex ratio of the emerging adults and egg-adult development time. Sangiovese presented a higher index, although it differed only from the Cabernet Sauvignon for the sex ratio (Table 7). When we observe the egg-adult development time, the Sauvignon Blanc and the Cabernet Sauvignon favored the development of *D. suzukii*, when compared to the Cabernet Franc.

In the free-choice bioassay with grapes without damage, where the flies had the possibility to choose the grape cultivar for oviposit, significant differences were observed for several variables analyzed (Table 7). Sangiovese obtained a higher number of puncture g^{-1} of grape, compared to the Chardonnay. For the number of eggs g^{-1} of grape, females emerged g^{-1} of grape, males emerged g^{-1} of grape and embryonic viability, Sangiovese grape presented higher indices and differed from the other cultivars (Table 7).

When an incision in the grape was made in non-choice bioassays, we observed differences among the treatments for the males emerged g^{-1} of grape, embryonic viability, and egg-adult development period. The Cabernet Franc presented the highest value of males emerged g^{-1} of grape but did not differ from the Cabernet Sauvignon. For the embryonic viability, the Cabernet Sauvignon proved to be superior to the others, differing significantly only from the Sangiovese (Table 7). Regarding the egg-adult period, the Cabernet Sauvignon was the one that presented the lowest indices differing significantly from the Sangiovese and Cabernet Franc (Table 7).

Regarding the grape chemical analysis, the °Brix and total titratable acidity values varied from 20.90 to 17.50, and 131.96 to 96.91 (Table 8). The pH values remained close to 3.0. Representing the physical analysis, the penetration resistance expressed in newtons (N) varied from 5.68 to 3.26 (Table 8). For the color patterns, the grapes for red wine presented *hue* angle values (h^o) close to 270º which corresponds to the blue/purple color, and the grapes for white wines presented values between 90 and 180º which correspond to the yellow and green colors, respectively. Regarding the luminosity, the grapes for red wine are closer to 0, which corresponds to the black/dark, than the grapes for white wine (Table 8).

Table 7 - Mean values (± standard error) per cultivar for the 2020/2021 season of São Joaquim Experimental Station vineyard of the Agricultural Research Company and Rural Extension of Santa Catarina. São Joaquim, Santa Catarina, Brazil

Cultivar	Punctures g^{-1}	Eggs g^{-1}	Females g^{-1}	Males g^{-1}	Embryonic viability (%)	Egg-Adult Time (days)	Sex ratio
Grapes with no damage and bioassay with non-choice							
Sauvignon Blanc	0.80±0.23 NS	0.44±0.15 NS	0.13±0.05 NS	0.10±0.04 NS	44.42±13.23 NS	14.43±01.27 a	0.63±0.07ab
Chardonnay	1.05±0.31	1.03±0.39	0.18±0.11	0.07±0.05	11.04±04.74	16.80±01.30 ab	0.74±0.08ab
Cabernet Franc	1.15±0.34	0.47±0.15	0.04±0.03	0.02±0.01	16.27±09.83	17.75±02.99 b	0.56±0.21ab
Sangiovese	0.78±0.19	0.66±0.15	0.10±0.02	0.02±0.01	21.46±06.14	16.12±02.83ab	0.79±0.07a
Cabernet Sauvignon	1.41±0.22	0.70±0.09	0.10±0.02	0.12±0.02	35.44±07.04	14.78±01.56 a	0.45±0.03b
p-value	0.3083	0.2511	0.2404	0.0499	0.3535	0.0059	0.0310
Grapes with no damage and bioassay with free-choice							
Sauvignon Blanc	1.14±0.37 ab	0.66±0.25 b	0.01±0.01 b	0.03±0.03 b	04.16±03.22 b	12.00±0.0 **	0.33±0.00**
Chardonnay	0.34±0.20 b	0.37±0.15 b	0.02±0.01 b	0.02±0.01 b	04.76±03.68 b	16.00±0.0	0.50±0.00
Cabernet Franc	0.66±0.11 ab	0.37±0.14 b	0.00±0.00 b	0.02±0.02 b	02.38±01.84 b	18.00±0.0	0.00±0.00
Sangiovese	1.69±0.19 a	2.20±0.30 a	0.34±0.06 a	0.72±0.19 a	40.70±08.14 a	15.40±01.81	0.38±0.08
p-value	0.03952	0.0008	0.0004	0.0124	0.0021	-	-
Grapes with damage and bioassay with non-choice							
Sauvignon Blanc	-	5.44±0.86 NS	1.47±0.23 NS	1.66±0.05 bc	64.21±22.17 ab*	14.00±00.48 ab	0.62±0.05 NS
Chardonnay	-	4.45±0.80	0.93±0.22	1.72±0.04 b	35.76±06.67 ab	14.16±00.46 ab	0.66±0.05
Cabernet Franc	-	7.02±1.29	1.56±0.31	2.28±0.11 a	41.14±04.42 ab	15.33±00.38 b	0.56±0.09
Sangiovese	-	5.07±0.68	0.82±0.21	1.30±0.10 c	20.87±03.13 b	15.83±00.42 b	0.80±0.06
Cabernet Sauvignon	-	5.27±0.42	1.83±0.20	2.01±0.12 ab	66.91±08.32 a	13.00±00.00 a	0.55±0.04
p-value		0.3194	0.0479	<0.0001	0.0392	0.0043	0.0733

Averages followed by the same letter, in the column in each bioassay, do not differ from each other according to a Tukey test at 0.05% of significance.
*The comparisons were performed in the Box-Cox transformed scale (ʎ = 0.06).
**Due to the low number of insects that emerged, it was not possible to perform the comparison.

Table 8 - Physicochemical analyses per wine grapes cultivar for the 2020/2021 season of São Joaquim Experimental Station of the Agricultural Research Company and Rural Extension of Santa Catarina. São Joaquim, Santa Catarina, Brazil

Cultivar*	ºBrix	TTA	pH	PR (N)	*h*º	L
Sauvignon Blanc	19.50	119.16	3.07	5.49	106.61	39.14
Chardonnay	17.50	96.91	3.00	3.77	100.58	40.74
Cabernet Franc	20.90	131.96	3.07	5.68	285.46	27.19
Sangiovese	19.30	108.76	3.16	3.23	257.90	27.31
Cabernet Sauvignon	19.40	126.02	3.27	3.26	267.72	27.68

TTA: total titratable acidity; pH: potential hydrogen; PR: penetration resistance; *h*º*: hue* angle; L: luminosity.
*It was not possible to perform the comparison of the cultivars, because only one compound sample was collected.

3.5 DISCUSSION

3.5.1 *Drosophila suzukii* monitoring

Although grape is not considered a good host for *D. suzukii*, the results obtained in this study reveal the constant presence of *D. suzukii* adults inside the vineyard and in the forest border that surrounds it. Similar results were also observed in vineyards in France (Delbac *et al.*, 2017; Rouzès *et al.*, 2012), Italy (Mazzetto *et al.*, 2015), Germany (Briem *et al.*, 2018; Weißinger *et al.*, 2019) and Turkey (Kasap; Özdamar, 2019). During the monitoring period in the vineyard located in São Joaquim, Santa Catarina, Brazil, the *D. suzukii* presence and activity were higher during the summer and early autumn, when the population peaks occurred. This is similar to what Briem *et al*. (2018) observed in vineyards located in Germany. This may have been influenced by the climate conditions favorable to the development of *D. suzukii*, besides corresponding to the maturation and harvest of the wine grapes.

In line with the results obtained in this study, Mazzetto *et al*. (2020) observed in vineyards located in Italy that at the beginning of the summer, the *D. suzukii* captures were low, but after the flowering, the population increased rapidly until the grape harvest period. Kasap, Özdamar (2019) observed in vineyards in Turkey that the *D. suzukii* population increases during the grape harvest, but the largest population peaks occurred after the harvest, indicating that *D. suzukii* feeds on fruits remaining in the vineyard or close to and continues its life cycle feeding on fruits such as nectarine and plum.

Small *D. suzukii* population peaks observed in September inside the vineyard and its surroundings can be related to the spring beginning. Adults go into diapause during the winter when the climate conditions are not favorable to their development, and return to their activities in the spring (Hamby *et al.*, 2016). Females can enter diapause while mated and have a greater survival through this period when compared to the males (Dalton *et al.*, 2011; Wiman *et al.*, 2014), favoring the reproduction and maintenance of the species in the vineyard. This explains how the September population peak was greater for females than for males. Rossi-Stacconi *et al*. (2016) state the importance of monitoring at the beginning of the winter since the number of females that enter diapause is an early predictor of the population size in the following summer.

When we observed the abundance of *D. suzukii* adults, we realized that the forest border that surrounds the vineyard is the preferred habitat when compared to the interior of the vineyards. Briem *et al*. (2018) also observed higher captures of *D. suzukii* adults in the forest border of the vineyards compared to inside the vineyards, where the number of adults captured at the peak season was above 1000 exemplars in the forest border and of 50 to 100 in the vineyards. Weißinger, Samuel, *et al*., (2019) also trapped fewer *D. suzukii* adults inside the vineyards than in the forest border with the presence of blackberries.

Some factors can favor the permanence of the adults in the forest border such as the microclimate, the availability of native and wild fruits, and a diversity of hosts. Briem *et al*. (2018) and Mazzetto *et al*. (2020) affirm that the areas that surround the vineyards can provide a habitat with favorable conditions for the reproduction and development of *D. suzukii*, and this may then lead to increased captures inside the vineyards. Drummond *et al*. (2019) report that native fruits serve as hosts, maintaining the *D. suzukii* population while there are no cultivated plants available in the field, until a susceptible culture is present, facilitating the seasonal fly migration.

The sex ratio indicated that the proportion of females in the total captured adults was greater in the forest border than inside the vineyards. These results draw attention to the crop protection of vines located close to the vineyard border since these could be at greater risk of attack by *D. suzukii* females that use the forest border as shelter. Kehrli *et al*. (2022) observed in the Switzerland vineyards that *D. suzukii* females place the largest number of eggs in berries in the interior of grape bunches close to the forest border, orchards, and gardens. This highlights the importance of the forest border as a source of survival and reproduction for the *D. suzukii*.

The temperature is a climate variable that contributes positively to *D. suzukii* population dynamics in orchards of several fruit plants (Wollmann *et al.*, 2019). In this study, we observed that the maximum temperatures (average 20 °C) and average (average 15 °C) contributed to the increase of the *D. suzukii* population in the forest border and inside the vineyard, respectively. However, Benito *et al*. (2016) affirm that high temperatures (>30°C) are limiting for *D. suzukii* propagation in Brazil. In this study, the average and maximum temperatures were not above 29°C, remaining within the limit for the development of *D. suzukii.* This proves that in the region of São Joaquim, Santa Catarina, Brazil, the temperature regime favors *D. suzukii* development.

Precipitation affected the population negatively, in agreement with what Manko *et al*. (2021) also observed in areas without commercial crops in Spain. There, precipitation combined with low temperature resulted in no capture of *D. suzukii*. According to Benito *et al*. (2016), a main factor that can limit the establishment of *D. suzukii* in Brazil is the relative humidity. Our findings showed that for this experimental vineyard, the relative humidity also disfavored the *D. suzukii* population. It may be that in this study there was an effect of the interaction of climate variables on the *D. suzukii* population, however, such interaction among the variables was not observed.

3.5.2 Wine cultivars susceptibility

According to others research, red wine grape cultivars were more susceptible to *D. suzukii* attack when compared to white wine grape cultivars (Ioriatti *et al.*, 2015; Linder *et al.*, 2014). In this study, both the red wine grape cultivars and the white wine grape cultivars proved susceptible to *D. suzukii* damage. However, in the test with free-choice, we observed significant differences among the cultivars, where the Sangiovese red wine grape presented itself as most susceptible to the *D. suzukii* attack when compared to the others. The Sangiovese grape susceptibility can be related to its subtle physicochemical characteristics.

The puncture rate of *D. suzukii* eggs in grapes depends, mainly, on the skin penetrating resistance (Entling *et al.*, 2019; Ioriatti *et al.*, 2015). Our results indicate that the Sangiovese presents less resistance to penetration than the Chardonnay since they differed significantly in the number of punctures received by the *D. suzukii* females. Although all cultivars received punctures, the Sangiovese grape was the one that presented higher numbers of eggs deposited in the grapes. This was also the cultivar with the lowest penetration resistance in the physicochemical analyses. According to Atallah *et al*. (2014), *D. suzukii* females can perform punctures without actually ovipositing. This observation draws attention to the management of *D. suzukii* in all cultivars, since the damage caused by the puncture results in cuticular lesions in the berries, which can facilitate the penetration of pathogens and yeasts responsible for the deterioration (Ioriatti *et al.*, 2015).

Subtle differences in the chemical composition of the fruits may influence the development of the larvae (Weißinger *et al.*, 2019). The higher performance for the number of females and males emerged, as well as the embryonic viability and sex ratio obtained in the tests with the Sangiovese can be hypothesized to be related to the chemical characteristics of the grape (°Brix, total titratable acidity and pH). These characteristics can provide improved nutritional substrate for larval development and consequently the emergence of adults. However, when we observed the performance of Sangiovese in non-choice bioassays for the grapes with artificial damage, the result showed lower emergence of adults and embryonic viability, indicating that the cultivar can differ in nutritional suitability depending on its physical integrity.

In grape susceptibility studies (wine and table grapes) cultivated in the Rio Grande do Sul highland region, Andreazza, Baronio, et al. (2016) observed that the Cabernet Sauvignon received *D. suzukii* punctures, however, did not lead to the emergence of adults. In this study, the Cabernet Sauvignon received punctures and successful development of immature phases of *D. suzukii* resulting in the emergence of adults. Possibly, slight differences in the experimental setup may have resulted in these different outcomes. Alternatively, these results could indicate that although both Rio Grande do Sul and Santa Catarina highland regions belong to the same temperate region of grape production, the intrinsic geoclimatic conditions of each sub-region, besides resulting in distinct '*terroir*' for the wines, can also influence in the cultivar susceptibility to the attack of pests.

The egg-adult development time of *D. suzukii* in non-choice bioassays without injury in the grapes was shorter in Cabernet Sauvignon (13 to 14 days) compared to Cabernet Franc (15 to 17 days). According to Andreazza, Bernardi, et al. (2016), the egg-adult life cycle of *D. suzukii* in an artificial specific diet for this species is 11 days at the used temperatures. Although the Cabernet Sauvignon provided shorter egg-adult development time compared to other grape cultivars, it was still longer than the *D. suzukii* development under optimal conditions. Again, the differences in development duration (days) among the wine cultivars for the egg-adult development time could be related to the intrinsic chemical characteristics of the cultivars. Still, the flies managed to oviposit and complete their biological cycle in the wine grapes cultivated in São Joaquim.

We also collected bunches of grapes during the 2020/21 season that had not been bagged in order to observe natural infestation. However, we did not observe any emergence of *D. suzukii* adults from these collected samples. Saguez, Lasnier, Vincent (2013) collected samples of grape bunches of Marechal Foch, Gamay, and Seyval Noir wine cultivars in Quebec, Canada, and observed that there was no emergence of *D. suzukii* adults. Similarly, (Rouzès *et al.*, 2012) did not observe *D. suzukii* infestation in wine grape bunches collected in Sauterner and Barsac vineyards in France, but the presence of *Drosophila melanogaster* and *Drosophila simulans*, as well as other drosophilids. So, natural infestation and full completion of the *D. suzukii* life cycle in grapes seems to be rare in vineyards, although the flies are detected inside the vineyards, accept grapes for oviposition, and could even complete their development in grapes.

Knowledge of the wine cultivars' susceptibility to *D. suzukii* attack is essential for the development of integrated management programs. These results, even if preliminary, can assist the winemakers of São Joaquim, SC, in the management of *D. suzukii*, indicating which cultivar the farmer should pay greater attention to with phytosanitary management. However, it is necessary to develop studies correlating the physicochemical characteristics of the grapes with the *D. suzukii* preference, for a better understanding of the behavior of this pest in the vineyards of São Joaquim, SC.

The constant presence of *D. suzukii* adults in the monitoring captures performed in this study, suggests that the population is already established in the vineyard. The high rates of adults captured in the forest border that surrounds the vineyard indicate that this habitat is preferred by flies, compared to the vineyard. Although *D. suzukii* adults prefer the forest border, it is not recommended in any way, to remove native vegetation or forests in the surroundings of the orchards, since they can also serve as shelter for numerous species of beneficial insects and natural enemies, besides contributing to the conservation of plant and animal species (Tscharntke *et al.*, 2007).

Despite not finding natural infestation in the wine grape cultivars evaluated in the vineyard, the fact that all the grapes offered to *D. suzukii* adults in bioassays in the laboratory presented puncture damage and eggs, suggests that all cultivars are under risk of *D. suzukii* attack. This highlights the need to develop field studies aimed at the integrated management of *D. suzukii*. Techniques such as toxic bait and mass trapping should be studied to evaluate their effectiveness and employability in the management of *D. suzukii* in the vineyards. These sustainable alternatives to conventional methods aim to control the insect-pest without the extensive use of insecticides.

3.6 CONCLUSION

D. suzukii population peaks occurred in the summer and at the beginning of the autumn, yet they were present year-round in the surrounding borders of the vineyard. Precipitation reduced *D. suzukii* population in the vineyard and the average and maximum temperature was correlated with population increase.

The Sangiovese cultivar was most susceptible to the attack and development of *D. suzukii* in free-choice bioassay, compared to the Sauvignon Blanc, Chardonnay, and Cabernet Franc. The Cabernet Sauvignon cultivar presented relatively rapid egg-adult development time in our bioassays. Although natural infestations appeared largely unsuccessful in the vineyards, our bioassays indicate that grapes can become infested and then contribute to the *Drosophila suzukii* population increase in the vineyards.

4 PHYSICAL-CHEMICAL CHARACTERISTICS OF WINE GRAPE CULTIVARS AND THEIR RELATION WITH SPOTTED-WING DROSOPHILA ARTIFICIAL INFESTATION

4.1 ABSTRACT

For effective pest management, it is valuable to understand the host preferences of the pest insects, and which factors determine this preference. The invasive pest, *Drosophila suzukii*, can infest a wide range of fruit crops. Grapes do not appear to be its preferred host, yet it can cause crop damage in vineyards. This work aimed to evaluate the susceptibility of grape wine cultivars to the invasive insect, *D. suzukii* in a laboratory and relate this to the physical-chemical propriety intrinsic to each cultivar. We performed non-choice tests, using undamaged and damaged grape berries of Chardonnay, Sauvignon Blanc, Sangiovese, Cabernet Franc, Cabernet Sauvignon, Merlot, and Montepulciano. Grapes were offered to *D. suzukii* adults in plastic-screened cages for 24 hours. Afterward, the number of punctures, eggs, and adults emerged, and the period of egg-adult development were evaluated. The physical characteristics of penetration resistance and color patterns, and the chemical characteristics of °Brix, pH, and total titratable acidity were evaluated. All the grape berries cultivars showed *D. suzukii* punctures and eggs were deposited. Sauvignon Blanc and Cabernet Sauvignon were shown to be most susceptible to *D. suzukii* infestation in both assays with undamaged and damaged grapes. Cabernet Sauvignon provided the best conditions for *D. suzukii* juvenile development. The penetration resistance is the main factor related to the reduction of female oviposition in undamaged berries, while pH is the main factor for damaged berries. Farmers should be aware of the susceptibility of Cabernet Sauvignon cultivar to the *D. suzukii* attack and consider adopting integrated pest management.

Keywords: *Drosophila suzukii*, fruit susceptibility, skin resistance, integrated pest management.

4.2 INTRODUCTION

The knowledge of host preference is crucial for effective insect pest management in the field, especially when the physical-chemical characteristics of fruits influence their susceptibility to pest damage (Lee *et al.*, 2011). *Drosophila suzukii* (Diptera: Drosophilidae) is a globally spread fruit fly species that is causing economic losses in fruit production. It has been extensively studied for its fruit preferences across various economic and non-economic plant species worldwide (Baena *et al.*, 2022; Burrack *et al.*, 2013; Liu *et al.*, 2019; Tait *et al.*, 2019). It is established that berries, characterized by dark color, soft skin, high sugar content, and low acidity, are most preferred by *D. suzukii* (Ioriatti *et al.*, 2015; Lee *et al.*, 2011). In this sense, cherry, blackberry, blueberry, strawberry, and raspberry are among the most preferred host crops for *D. suzukii*.

During the past 1.5 decades, *D. suzukii* became worldwide known as a pest due to its potential to cause economic losses through direct and indirect damage to the fruit (Tait *et al.*, 2021). Unlike the other drosophilids, the female of *D. suzukii* has a serrated ovipositor, enabling her to puncture the skin of fruits to lay eggs inside fruits. As the larvae develop within the fruit, they consume the pulp, rendering it unmarketable (Funes *et al.*, 2018). The small perforations created during egg-laying also provide sites for pathogens infections, accelerating fruit decay (Ioriatti *et al.*, 2018). Females of *D. suzukii* can lay 7 to 16 eggs per day and they may lay up around 400 eggs throughout their lifespan (Cini; Ioriatti; Anfora, 2012).

Among the *D. suzukii* hosts, the grape susceptibility is a bit controversial. In Japan, grapes are considered suitable hosts for *D. suzukii* (Kanzawa, 1939). In Thompson grapes and other red cultivars cultivated in the state of California, U.S.A., *D. suzukii* females were able to pierce the skin, but in most cases, they were unable to lay eggs ((Atallah *et al.*, 2014). Other studies have shown that the fly oviposits a smaller number of eggs in white table grapes ('Thompson'), and the developmental time of juveniles is lengthened and survival less, compared to larvae that develop in other fruits, such as raspberries and cherries (Lee *et al.*, 2011). Larvae of *D. suzukii* were found feeding on wine grapes grown in the province of Trento, Italy, resulting in a negative impact such as cracking, disease, and attraction of other animals during the harvest period ((Ioriatti *et al.*, 2015).

Adults of *D. suzukii* can complete their life cycle on both table and wine grapes, but both are not considered good hosts (Bellamy; Sisterson; Walse, 2013; Brilinger *et al.*, 2023; Wang; Kaçar; Daane, 2019).

The presence of *D. suzukii* was identified in monitoring traps in vineyards in the Sauternes and Barsac region of France in 2011, but the presence of larvae in the fruits was not observed (Rouzès *et al.*, 2012). In northern Italy in 2011, in vineyards of the cultivar Schiava, the presence of *D. suzukii* larvae was detected inside the grapes. The damage caused by egg-laying and the presence of larvae caused an increase in the incidence of pathogens (Sinn; Consulenza, 2011). In Quebec, Canada, and Michigan U.S.A., the presence of larvae was detected in red grapes (Saguez; Lasnier; Vincent, 2013; Van Timmeren; Isaacs, 2014).

In wine grapes, the damage can also affect and reduce the quality of the must, material used to make juices and wines. Oviposition of *D. suzukii* in wine grapes increased during the ripening period, being mainly related to the decrease in the skin's resistance to ovipositor penetration, increased sugar content, and decreased pH of the fruits (Ioriatti *et al*., 2015). The susceptibility of wine grapes to *D. suzukii* varies over time, since the varieties mature at different times in the same harvest period (Shrader; Burrack; Pfeiffer, 2019).

In the south of Brazil, research carried out by Brilinger *et al*. (2023). showed that *D. suzukii* is present inside the vineyards during the grape ripening and additionally, it was identified that some grape cultivars can be susceptible to *D. suzukii* attack. To follow up on these observations, the work presented in this chapter aimed to investigate the susceptibility of wine grape cultivars to *D. suzukii* damage in the laboratory and relate it with the physical-chemical properties intrinsic to each cultivar.

4.3 METHODOLOGY

4.3.1 *Drosophila suzukii* rearing conditions

The insects used in the bioassays came from the *D. suzukii* culture from the Entomology Laboratory of the Agroveterinary Sciences College of the Santa Catarina State University (CAV/UDESC). They were maintained at a temperature of 25 ± 2 ^{o}C, RH 65 ± 10%, and a 12 h photophase in screened plastic cages (28 x 28 x 28 cm). The insect culture was started in March 2019 with *D. suzukii* pupae that were kindly provided by the Entomology Laboratory of Temperate Agriculture station of Brazilian Agricultural Research Corporation (Embrapa), Pelotas, RS, Brazil. The adults were fed with an artificial diet based on corn flour 80 g, yeast 40 g, sugar 100 g, agar 8 g, propionic acid 3 mL, Nipagin 8 mL in a dilution of 10% alcohol, and 1 L of distilled water (Schlesener *et al.*, 2018).

4.3.2 Susceptibility of wine cultivars bioassays

The grapes used in this study were obtained from the vineyard of the São Joaquim Experimental Station (EESJ) of the Santa Catarina Agricultural Research and Rural Extension Company (EPAGRI) located in the municipality of São Joaquim, Santa Catarina, Brazil, as described in Brilinger *et al*. (2023). In the crop season of 2021/22, bunches of the wine grape cultivars were bagged in the field with TNT tissue bags (non-woven fabric), at the "*veraison*" phenological stage, to prevent natural infestation by *D. suzukii*. At the harvest, those bunches were collected. During the entire grape maturation period, no insecticides were applied in the vineyard.

No-choice bioassays with undamaged grapes and damaged grapes were carried out at the Entomology Laboratory of CAV/UDESC, under temperature conditions of 25 ± 2 ^{o}C, RH 65 ± 10%, and 12 h photophase, following the methodology described by Ioriatti *et al*. (2015) and adapted by Brilinger *et al*. (2023). In all bioassays, insects were deprived of the artificial diet for 12 hours before the bioassays were performed. The experimental units consisted of five-day-old *D. suzukii*, four females and one male, in cages built with transparent plastic pots (500 mL), with a hollow bottom and closed with voile fabric to prevent insects from escaping and placed in a plastic lid (11 cm ø) lined with filter paper.

Bioassay using undamaged grapes – seven wine grape cultivars were tested: Chardonnay, Sauvignon Blanc, Cabernet Sauvignon, Cabernet Franc, Sangiovese, Merlot, and Montepulciano. Ten fully intact grapes of the above grape varieties were inserted into each cage. The bioassay was carried out in a completely randomized design with respect to cultivars assigned to cages, with ten replications per treatment.

For the bioassay using damaged grapes, five wine grape cultivars were tested: Chardonnay, Sauvignon Blanc, Cabernet Sauvignon, Cabernet Franc, and Sangiovese. Artificial damage was made by an incision with a scalpel, sterilized in 99% ethanol after each incision, with a cut of approximately 1 cm in the longitudinal length of the grape. The bioassay was carried out in a completely randomized design, with six replications per treatment.

In all bioassays, the four *D. suzukii* females and one male were kept in cages containing the grapes for 24 hours of exposure. Afterward, the adults were removed from the cages and the number of punctures and eggs were counted with the aid of a stereoscopic binocular magnifying glass with 5x magnification (Eyemag, Carl-Zeiss). Grapes containing *D. suzukii* eggs were stored in plastic containers and maintained at 25 ± 2 ^{o}C, RH 65 ± 10%, and 12 hours photophase. Daily observations were made to record adult emergence for 25 days after the start of the assay. From the collected data, we estimated embryonic viability and the period of egg-adult development. The sex ratio was calculated from the emerged adults, using the formula: number of females/(number of males + number of females) (Silveira-Neto *et al*., 1976).

4.3.3 Physical-chemical analysis

To perform the physical-chemical analysis, samples of each grape cultivar were composed of 10 grapes. For the chemical characteristics, the soluble solids content (°Brix), total titratable acidity (TTA), and pH were measured. The soluble solids content (°Brix) was measured with the aid of a bench portable digital refractometer. The total titratable acidity was obtained by the titration of a grape juice sample, diluted in distilled water, and titrated with NaOH 0.1 N solution, through an automatic titrator. The pH was measured directly in the grape juice using a pH Meter calibrated at 6.86 and 4.01. The physical characteristics analyzed were penetration resistance (PR) and color patterns. The penetration resistance was determined in newton (N), using a digital penetrometer, with a tip of 2 mm and penetration of 10 mm, in the equatorial zone of the fruit. The skin background color was determined by an electronic colorimeter, through the realization of readings in the equatorial region of the fruits and expressed in terms of hue angle (h^{o}) (0° = red; 90° = yellow; 180° = green and 270° = blue) and luminosity (L) that refers to the scale that varies from black (0) to white (100).

4.3.4 Data analysis

The variance analysis model was used to analyze the data of the *D. suzukii* oviposition. The assumptions of normality and homogeneity of variances were verified and when one of the assumptions was not met, the transformation with Box-Cox optimal lambda was used (Venables; Ripley, 2002). In cases where even after data transformation the model assumptions were not met, a generalized linear model was used. For the count data, the Poisson, quasi-Poisson, and negative Binomial distribution were used, and for the proportion data, the Binomial and quasi-binomial distribution were used. For diagnostic analyses, seminormal graphs with simulation envelopes were used (Moral; Hinde; Demétrio, 2017). Principal component analysis was used for the physical-chemical analysis data and the results were represented using Biplot with the aid of the "ggbiplot" package (Vu, 2011). All analyses were carried out in the R environment (R Core Team, 2021) considering a 5% level of significance.

4.4 RESULTS

4.4.1 Susceptibility of wine cultivars

The results from both assays, with undamaged and artificially damaged grapes, indicate that all grape cultivars are susceptible to *D. suzukii* oviposition, as evidenced by the presence of punctures and eggs (Table 9). Comparing the numbers of eggs laid on each grape cultivar when provided as undamaged or damaged grapes, undamaged berries received fewer eggs than those with artificial damage (Table 9). Also, the embryonic viability results differed between the damaged and undamaged grapes, but not always in the same manner (Table 9). The sex ratio of the *D. suzukii* adults emerged was the only biological parameter that did not show statistical differences between the grape cultivars, in either of the assays (Table 9).

Among the grape cultivars without artificial damage, Cabernet Sauvignon had the highest number of punctures, although it did not receive the highest number of eggs. Chardonnay grape, on the other hand, had the highest egg numbers (Table 9). Cabernet Sauvignon and Chardonnay did not differ from each other for punctures and egg parameters.

Table 9 - Mean values (± standart error) of *Drosophila suzukii* biological parameters per grape wine cultivar for the 2021/2022 crop season

Cultivar	Punctures g^{-1}	Eggs g^{-1}	Female g^{-1}	Male g^{-1}	Embryonic Viability (%)	Period Egg-Adult (days)	Sex rate
Undamaged berries							
Merlot	0.80±0.17 b	0.27±0.11 b	0.01±0.01 b	0.01±0.01 b	01.32±1.32 ab	20.00±0.00 a*	0.50±0.00*[NS]
Sauvignon Blanc	0.94±0.11 b	0.83±0.08 a	0.08±0.02 ab	0.12±0.04 a	23.00±5.93 ab	19.25±0.86 a	0.41±0.10
Chardonnay	1.21±0.13 ab	0.90±0.12 a	0.07±0.02 ab	0.11±0.03 a	22.49±3.65 b	20.30±0.70 a	0.34±0.09
Cabernet Sauvignon	1.80±0.17 a	0.78±0.14 a	0.18±0.03 a	0.15±0.04 a	43.01±4.55 a	19.70±0.92 a	0.62±0.06
Cabernet Franc	0.78±0.18 b	0.13±0.04 b	0.00±0.00*	0.00±0.00*	00.00±0.00*	00.00±0.00*	0.00±0.00*
Sangiovese	0.98±0.14 b	0.86±0.12 a	0.10±0.02 ab	0.09±0.03 ab	22.47±4.73 b	16.10±0.23 b	0.62±0.08
Montepulciano	0.19±0.04 c	0.11±0.03 b	0.00±0.00*	0.00±0.00*	00.00±0.00*	00.00±0.00*	0.00±0.00*
p-value	<0.0001	<0.0001	<0.0001	<0.0002	0.0066	0.0001	0.1221
Damaged berries							
Sauvignon Blanc	-	4.31±0.44 a	0.39±0.12 b	1.70±0.07 b	16.65±04.36 bc	22.00±0.73 a	0.53±0.04 [NS]
Chardonnay	-	4.77±0.55 a	0.37±0.16 b	1.97±0.05 a	12.20±06.73 c	22.00±0.73 a	0.85±0.10
Cabernet Sauvignon	-	3.95±0.58 a	1.56±0.27 a	1.89±0.02 ab	89.77±31.45 a	17.00±0.00 c*	0.56±0.05*
Cabernet Franc	-	3.87±0.57 a	1.05±0.32 ab	1.91±0.10 ab	56.91±15.93 ab	19.17±0.65 b	0.57±0.12
Sangiovese	-	1.06±0.19 b	0.29±0.07 b	0.92±0.05 c	59.76±10.68 ab	15.83±0.54 c	0.46±0.03
p-value		0.0001	0.0021	<0.0001	0.0004	<0.0001	0.1929

* The data were excluded from the analysis because they presented only one record or no record in the treatment. The comparison with the other treatments was carried out based on the confidence interval of the other treatments.

When considering embryonic viability, Cabernet Sauvignon proved to be the most favorable for *D. suzukii* juvenile development (Table 9). In contrast, the Montepulciano grape showed the lowest number of punctures and eggs and did not support *D. suzukii* development, as evidenced by the absence of adult emergence (Table 9). The Cabernet Franc grape received punctures and eggs but also did not provide conditions that led to successful adult emergence (Table 9).

Artificially damage caused to the grapes revealed interesting insights into the oviposition preferences and larval development of *D. suzukii* among the grape cultivars. The number of eggs that the damaged grapes received was generally higher than in the undamaged grapes of the same cultivar. Chardonnay received the highest number of eggs, yet it did not promote favorable conditions for juvenile development, with only a 12% emergence of adults (Table 9). In contrast, the Sangiovese, despite being less preferred for oviposition, demonstrated good conditions for larval development, with embryonic viability reaching almost 60%. Once again, Cabernet Sauvignon emerged as one of the most preferable grape cultivars for oviposition, providing optimal conditions for larval development, with an embryonic viability of 89% (Table 9).

4.4.2 Physical-chemical analysis

The chemical analyses of the grapes as °Brix, pH, and total titratable acidity values showed an average of 19.38, 3.04, and 124.34 mEq/L, respectively (Table 10). In the physical analyses, penetration resistance expressed in newtons (N) ranged from 3.19 to 6.37 (Table 10). For color patterns, the red wine grapes presented hue angle (h^o) values between 200 and 290º, which correspond to the blue/purple color, and the white wine grape presented values between 90 and 180º, which correspond to the colors yellow and green, respectively. As for luminosity, Merlot and Cabernet Sauvignon grape are closer to 0 than Chardonnay, which corresponds to black/dark (Table 10).

Principal component analysis revealed distinct behaviors among the grape wine cultivars tested in this study (Figure 11). In the assay with undamaged grapes, the grape cultivars Cabernet Sauvignon, Sauvignon Blanc, and Chardonnay exhibited positive values for the first principal component (PC1), while Merlot, Montepulciano, Sangiovese, and Cabernet Franc displayed negative values. Regarding the second principal component (PC2), Sangiovese, Cabernet Sauvignon and Chardonnay showed positive values, while Cabernet Sauvignon and Merlot, Montepulciano, Cabernet Franc, and Sauvignon Blanc exhibited negative values (Figure 11A).

The first two principal components explained 64.02% of the data variability for undamaged berry assay (Figure 11A). A negative correlation was observed between the variable penetration resistance and punctures g^{-1}, adults g^{-1}, and embryonic viability since they are in opposite directions in the biplot (Figure 11A). Specifically, the cultivars Chardonnay and Cabernet Sauvignon showed a strong association with adults g^{-1}, and Cabernet Franc was associated with the highest penetration resistance (Figure 11A). On the other hand, the highest values of total titratable acidity, °Brix content were associated with Sauvignon Blanc, and pH was linked with Sangiovese, and hue angle was associated with Merlot. The grape cultivar Montepulciano is associated with both hue angle and penetration resistance (Figure 11A).

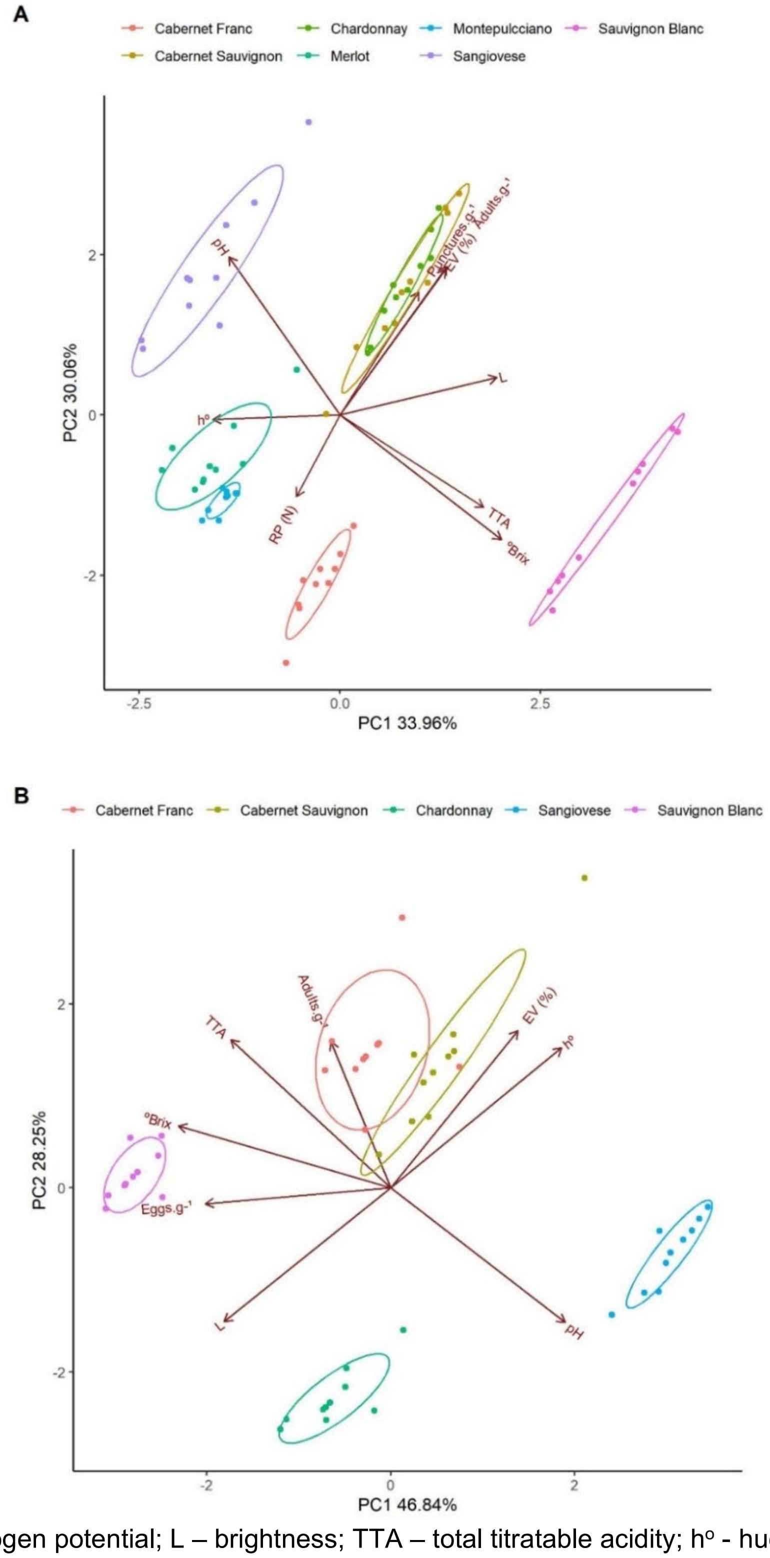
Table 10 - Physical-chemical analyses by grape wine cultivar for the 2021/2022 crop season

Cultivar*	°Brix	TTA	pH	RP (N)	*h°*	L
Merlot	19.00	101.85	3.03	5.09	202.96	26.62
Chardonnay	19.10	101.85	3.10	3.51	104.49	40.84
Cabernet Sauvignon	19.00	140.48	3.05	5.28	270.91	32.27
Cabernet Franc	19.90	132.58	2.92	6.37	280.78	30.57
Sangiovese	18.30	102.73	3.14	3.19	286.71	28.69
Sauvignon Blanc	21.24	161.81	2.91	3.49	115.49	37.73
Montepulcciano	19.10	129.07	3.06	4.92	271.31	29.14

TTA: total titratable acidity in mEq/L; pH: hydrogen potential; RP: penetration resistance; *h°: hue* angle; L: luminosity. *It was not possible to perform a comparison among the cultivars, because only one compound analysis was performed per cultivar.

In the assay with artificial damage to the grape, the variables penetration resistance and the parameter punctures g^{-1} were excluded from the principal component analysis. Here, the first two principal components explained 75.09% of the data variability (Figure 1B). The grape cultivars Sangiovese and Cabernet Sauvignon showed positive values for the first principal component (PC1) and Chardonnay, Sauvignon Blanc, and Cabernet Franc displayed negative values. For the second main component (PC2), the cultivars Sauvignon Blanc, Cabernet Franc, and Cabernet Sauvignon showed positive values, whereas Sangiovese and Chardonnay exhibited negative values (Figure 11B).

Figure 11 - Biplot PC1 versus PC2 correlating the physical-chemical characteristics of each grape wine cultivar with the biological aspects of Drosophila suzukii evaluated in the susceptibility assay with undamaged berries (A) and damaged berries (B)



Note: pH – hydrogen potential; L – brightness; TTA – total titratable acidity; h° - hue angle; RP (N) – penetration resistance in Newton; Adultos.g^{-1} – number of adults emerged per gram of grapes; Punctures.g^{-1} – number of punctures per gram of grape; and EV – embryonic viability.

A negative correlation was observed between the variable pH and total titratable acidity, as well as between hue angle and luminosity, as they are positioned in opposite directions in the biplot (Figure 11B). The grape cultivar Cabernet Franc is associated with adult g^{-1}, while the Cabernet Sauvignon is associated with both adult g^{-1} and embryonic viability (Figure 11B). On the other hand, °Brix content and eggs g^{-1} are associated with Sauvignon Blanc, while pH is associated with Sangiovese (Figure 11B).

4.5 DISCUSSION

In this study, we evaluated the susceptibility of the wine grape cultivars to *Drosophila suzukii* infestation. In bioassay using undamaged and artificially damaged grape berries, we assessed the susceptibility of the cultivars Cabernet Sauvignon, Merlot, Chardonnay, Cabernet Franc, Sauvignon Blanc, Sangiovese, and Montepulciano. In non-choice assays in the laboratory, all the grape wine cultivars exhibited susceptibility to *D. suzukii* oviposition. It was clear that *D. suzukii* can lay eggs not only in healthy grapes but also in damaged grapes. To better understand the preference of *D. suzukii* for certain grape cultivar, we related the susceptibility data to the physical-chemical characteristics of each cultivar. The results demonstrate that subtle differences in the physical-chemical composition of each cultivar can lead to different susceptibility rates. Indeed, penetration resistance was demonstrated to be the principal characteristic of reducing *D. suzukii* infestations for undamaged grape berries, while the pH was correlated with a reduction in adult emergence in grape berries with artificial damage.

The color patterns and luminosity were not related to *D. suzukii* artificial infestation, which was shown from the high rates of oviposition both on red and white grapes (Cabernet Sauvignon and Chardonnay). Differently, Linder *et al*. (2014) observed that red grape cultivars were more attractive to *D. suzukii* than white grapes. Also, Weißinger *et al*. (2019) state that the *D. suzukii* infestation was almost exclusively to red cultivars. However, our results showed that the color did not matter for *D. suzukii* infestation in artificial non-choice bioassays. The penetration resistance was related to reduced *D. suzukii* oviposition in the bioassay with undamaged grape berries, where Cabernet Sauvignon and Chardonnay seemed to be the grape wine cultivars with the highest susceptibility, while Montepulciano was less susceptible. Our findings reinforce the statement made by Baser *et al*. (2018), Entling *et al*. (2019), Ioriatti *et al*. (2015), Pelton, Gratton, Guédot (2017), and Shrader *et al*. (2019) that for the grapes, the penetration resistance is the best predictor of *D. suzukii* oviposition and it can be used as an indicator of infestation risk.

When comparing the number of eggs laid by *D. suzukii* females in no-choice bioassays between undamaged and damaged grapes, it was clear that the skin is a barrier, and the oviposition increases when the berry skin is damaged. Our findings agree with the results of Linder *et al*. (2014), Pelton, Gratton, Guédot (2017), and Weißinger *et al*. (2019) that showed, both in no choice-tests and choice tests, *D. suzukii* females lay considerably more eggs on grapes with damage than undamaged grapes. The grape with skin damage allows direct and easier access to *D. suzukii* oviposition into the grape pulp, turning unsusceptible cultivars into suitable hosts (Weißinger *et al.*, 2019). In this study, the number of eggs was on average 5 times higher in damaged berries than undamaged ones for Cabernet Sauvignon, Sauvignon Blanc, and Chardonnay, 9 times higher for Sangiovese, and even 29 times higher for Cabernet Franc. Thus, the preservation of the health of the berry skin might play an important role in avoiding *D. suzukii* oviposition in grape wine (Entling *et al.*, 2019). The increase in egg-laying by *D. suzukii* and survival in damaged grape berries indicates that *D. suzukii* can use this compromised material as a nutrient source and increase the population in the field (Ioriatti *et al.*, 2015). Due to this, it is important to avoid damage either mechanical or animal such as birds and bees, on the grapes in the field.

On the other hand, Andreazza *et al*. (2016) state that physical characteristics, e.g. grape skin resistance, alone cannot account for differences in grape susceptibility, and other chemical parameters may play an important role in *D. suzukii* oviposition as well. Similarly, Ioriatti *et al*. (2015) observed that the *D. suzukii* oviposition in undamaged berries increased as sugar contents increased and pH decreased. In this study in undamaged grapes, the chemical characteristic did not show an influence on the number of either punctures or eggs by *D. suzukii* females. However, when the skin was not serving as a barrier, in the bioassay with artificially damaged grapes, the chemical characteristics pH, total titrable acidity, and °Brix (sugar content) were demonstrated to be important to *D. suzukii* oviposition. Entling *et al*. (2019) observed that tartaric acidity had influenced the *D. suzukii* oviposition in grapes, whereas sugar content and volatile acidity had no effect. Differently, Baser *et al.* (2018) found that grapes cultivated under an organic system promoted a higher rate of sugar content exposing the grapes to heavier attack by *D. suzukii*. The results of our study showed that pH can reduce oviposition, as well as reduce the number of adults that emerged, while °Brix was positively related to the number of eggs. The latter may be playing a role as an attractant to *D. suzukii* oviposition. The total titrable acid was related to adult emergence, thus can be favoring the development of *D. suzukii* juveniles.

The susceptibility results obtained in this study were somewhat different from those that we observed in the previous study (Brilinger *et al.*, 2023), and this applies to the physical-chemical characteristics as well. This suggests that the susceptibility of the cultivars can change depending on the physical-chemical composition – *terroir* – of the grape wine in each season. Weißinger *et al*. (2019) observed that the field infestation by *D. suzukii* varies strongly within and across years, being explained by the seasonality in environmental conditions changes. Also, not all eggs developed and turned into adults, which can be related to the injury caused by the oviposition and bacterial infections according to Ioriatti *et al*. (2015), or because most of the cultivars did not provide good conditions for the *D. suzukii* immature development. On the other hand, the higher rate of embryonic viability presented by the Cabernet Sauvignon in both assays suggests that this grape cultivar can provide suitable conditions for *D. suzukii* larval development, contributing to the population increase of this species population in the field. Likewise, Baser *et al*. (2018) observed that table grapes provided a suitable substrate for *D. suzukii* full development, suggesting that table grape is potentially a profitable host.

4.6 CONCLUSION

The knowledge of grape wine susceptibility plays an important role in *Drosophila suzukii* management and can therefore limit economic losses and improve the management in the field. Our results indicate that *D. suzukii* oviposits and develops in adults on Cabernet Sauvignon, Chardonnay, Sauvignon Blanc, Cabernet Franc, Merlot, and Sangiovese cultivars and that skin penetration resistance is the principal characteristic to reduce *D. suzukii* infestation. This implies that the skin works as a barrier. Thus, when the grape berry has no damage, there are no strong concerns of *D. suzukii* as a primary pest, because it is difficult for the females to oviposit. However, when the grapes are near the harvest period, it is important to avoid berry skin damage as it can attract and favor *D. suzukii* oviposition and consequently increase population growth. The withe grape cultivars Chardonnay and Sauvignon Blanc showed to be particularly susceptible to *D. suzukii* infestation, in both assays, as well as Cabernet Sauvignon. Taking into account the grape maturation period, Cabernet Sauvignon is the one to be most susceptible to *D. suzukii* attack, because it has a long maturation period, being one of the last wine cultivars to be harvested. In contrast, Chardonnay and Sauvignon Blanc have a short maturation period and are harvested earlier. Therefore, we recommend that the farmers be vigilant, especially in Cabernet Sauvignon vineyards. When they are aware when *D. suzukii* moves into the vineyard, they can take appropriate management steps to avoid production losses.

5 EXPLORING MATRINE AND OXYMATRINE AS POTENTIAL BIOINSECTICIDE TO CONTROL *Drosophila suzukii*

5.1 ABSTRACT

A bottleneck in sustainable fruit production is the replacement of chemical products with natural compounds. Matrine and oxymatrine, derived from a Chinese medicinal herb, have exhibited potential for controlling agricultural pests. However, there is no information regarding their effectiveness in managing dipterans. Thus, we carried out experiments to evaluate the effect of the botanical compounds matrine and oxymatrine on *D. suzukii* adults under laboratory conditions. First, we tested four doses of the commercial product matrine, with distilled water as a control treatment. Each treatment was offered as a droplet, to 5 *D. suzukii* couples in screened cages. Adult mortality was evaluated at intervals of 12, 24, 36, 48, 60, and 72 hours post-exposure. All doses tested of the commercial matrine-based product caused up to 58% of mortality in *D. suzukii* adults. Second, the consumption of pure extract of oxymatrine was evaluated using a CAFE assay. Three *D. suzukii* females were exposed to oxymatrine concentrations of 0.05, 1.0, 2.5, 5.0, and 7.0% in a 20% sugar solution for 24 hours. Afterward, the flies were deprived of food for 40 hours, and mortality was evaluated. The pure extract of oxymatrine induced mortality in *D. suzukii* females only at doses of 2.5, 5.0, and 7.0%. These botanical compounds demonstrate the potential to be used to control *D. suzukii* adults and can be explored as one tool of the Integrated Management Program.

Keyword: botanical insecticide, spotted-wing drosophila, integrated pest management, organic fruit production.

5.2 INTRODUCTION

The adverse effects of insecticides on human health, the environment, pollinators, and other beneficial insects, are well documented (Ara; Haque, 2021; Januário *et al.*, 2021). Part of these can be attributed to inappropriate application, but even with appropriate application, an impact on the environment and non-target species is unavoidable. Additionally, there is a growing concern about the accelerated development of insecticide resistance (Chagnon *et al.*, 2015; Januário *et al.*, 2021; Smirle *et al.*, 2017). Consequently, the research for the development and registration of insecticides with low toxicity for mammals and good effectiveness for pest management is ongoing. Botanical compounds offer several advantages over synthetic molecules. They are derived from renewable resources and often quickly degrade in the ecosystem, due to their high volatility. Additionally, they are often less toxic, and their toxicity tends to be species-specific with fewer effects on non-target species (Leahy *et al.*, 2014). Furthermore, the risk of accelerating resistance in insects is reduced when using botanical compounds, since they usually consist of complex mixtures of bioactive components with different modes of action (Giunti *et al.*, 2022; Jang *et al.*, 2017).

Matrine is a new, broad-spectrum botanical compound that has gained attention as an insecticide for controlling pests in crops (Celiz; Ubaub, 2019; Zanardi *et al.*, 2015). It is a quinolizidine alkaloid derived from the medicinal herb native to Asia, *Sophora flavescens*. Additionally, the secondary metabolites sophoridine, sophocarpine, cytisine, and aloperine also show antagonistic activity at nicotinic acetylcholine receptors of insects (Huang, Xu, 2016; Liu et al., 2008). Insects treated topically with matrine exhibit a rapid onset of sluggish behavior and paralysis, without evidence of hyperexcitation (Bloomquist *et al.*, 2018).

Matrine compounds have been found to inhibit HMG-CoA reductase, a key enzyme responsible for regulating the biosynthesis of insect juvenile hormones (Xu *et al.*, 2020). Matrine showed a reduction in excitatory post-synaptic potentials through electrophysical assay in housefly (*Musca domestica*) larvae and reduced the firing of *Drosophila melanogaster* larval central neurons (Bloomquist *et al.*, 2018). When used in combination with the entomopathogenic fungus *Lecanicillium muscarium*, matrine has shown synergistic effects in *Bemisia tabaci*, leading to changes in enzymatic activities (Ali *et al.*, 2017). Anti-feeding/repellency action was also identified as a matrine effect in multiple insects such as flower thrips and Asian citrus psyllid, and *Orius laevigatus*, although it proved to be harmless to this insect predator (Celiz; Ubaub, 2019; Kordestani *et al.*, 2022; Zanardi *et al.*, 2015).

Commercial products based on matrine are already available for managing agricultural pests. In China, for instance, Baicao® n1 (Beijing Multigrass Formulation Co., Ltd) is for sale. In Iraq and some countries of Africa, the commercial product Levo

2.4 SL (Prosuler oxymatrine 2.4%) (UPL Ltd) is commercially accessible for agricultural pest control. Likewise, in Brazil, a commercial product known as Matrine® (manufactured by Inner Mongolis Kingbo Biotech Co., Ltd and imported by Dinagro Agropecuária Ltda.) is legally allowed to be used in organic fruit production to manage pests like the oriental moth (*Grapholita molesta*) and mites (*Tetranychus urticae*, *Brevipalpus phoenicis*, *Phyllocoptruta oleivora*) (MAPA, 2023). On the other hand, in Europe, there is a commercial liquid fertilizer product named Deffort, which combines 8% *Sophora flavescens* with 1% magnesium and 1% zinc. While it is recommended as a liquid fertilizer, it has also been tested for its insecticidal effects (Gargani *et al.*, 2013).

In general, conventional insecticides remain the first option to control fruit flies, including *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), in fruit production (Shawer, 2020). Commonly known as the spotted-wing drosophila (SWD), *D. suzukii* stands apart from other *Drosophila* species due to its serrated ovipositor. This unique characteristic allows for puncturing the fruit skin and oviposition in healthy fruits, rendering the fruits unmarketable (Garcia, 2020). Due to its invasive potential and high ability to cope with new environments, as well as its potential to cause economic losses, *D. suzukii* is a threat to fruit crop production systems worldwide (Haye *et al.*, 2016). The management and control of *D. suzukii* is challenging, due to its wide host range, continuous availability of fruits, and the rapid and short biological cycle, which intensifies the multiplication and population growth of the species (Walsh *et al.*, 2011). Currently, many farmers rely on conventional insecticides for the control of this pest, such as pyrethroids, carbamates, and organophosphates (Van Timmeren; Isaacs, 2013). However, this is not sustainable and resistance against these insecticides may eventually evolve. Consequently, there is an urgent need for the development of effective, yet sustainable, tools to manage this species in the field.

To investigate whether matrine could be an alternative insecticide, suitable for *D. suzukii* management, we want to assess the toxicity of the insecticide matrine/oxymatrine towards adult *D. suzukii*. Exposing adults to the commercial matrine-based insecticide and the pure extract of oxymatrine on *D. suzukii* adults under laboratory conditions, we evaluated mortality and quantified the consumption of oxymatrine solution using a Capillary Feeder (CAFE) assay.

5.3 MATERIAL AND METHODS

5.3.1 Commercial matrine-based insecticide assay

The adult insects used in the bioassays came from the *D. suzukii* reared at the Entomology Laboratory of the Agroveterinary Sciences Center of the Santa Catarina State University (CAV/UDESC), at a temperature of 25 ± 2 ^{o}C, RH 65 ± 10% and a 12 h photophase in screened plastic cages (28 x 28 x 28 cm). The insect culture was started in 2019 with *D. suzukii* pupae, kindly provided by the Entomology Laboratory of Embrapa Clima Temperado, Pelotas, RS, Brazil. The adults were fed with an artificial diet based on corn flour (80 g), yeast (40 g), sugar (100 g), agar (8 g), propionic acid (3 ml), Nipagin (8 ml, in a dilution of 10% alcohol) and 1 L of distilled water (Schlesener *et al.*, 2018).

The commercial product used in this assay is composed of an ethanolic extract of *Sophora flavescens,* containing matrine (19.05%) and other ingredients (80.95%); it is available in the Brazilian market as Matrine® (Dinagro Agropecuária, São Paulo, Brazil). This dose is equivalent to 0.2% oxymatrine. Four Matrine® doses were tested: i) 0.8 ml L^{-1} water; ii) 1.0 ml L^{-1} water; iii) 1.2 ml L^{-1} water; and iv) 1.4 ml L^{-1} water. These tested dose ranges were based on the recommendation provided by the manufacturer. Distilled water was used as a control. The bioassays were carried out three times (triplicate) in a controlled environment with a temperature of 25 ± 2 ^{o}C, RH 65 ± 10%, and a 12 h photophase.

Before to the bioassay, five-day-old *D. suzukii* males and females were maintained under starvation conditions for 12 hours. Each experimental unit consisted of screened cages built with transparent plastic pots (500 mL), with a hollow bottom and closed with voile. In each cage, five *D. suzukii* couples were released and one drop of 20 µL of the insecticide solution deposited on an acrylic film (1 cm^2) was offered at the cage bottom. After two hours of exposure, cotton wool soaked in a 20% sugar solution was introduced into the cages. Adult mortality was evaluated at 12, 24, 36, 48, 60, and 72 hours after exposure to the insecticide doses. Insects that showed no reaction to touch with fine-tipped brush bristles were considered dead. For each of the replications, a completely randomized experimental design was adopted with respect to doses assigned to cages, with 10 replications per treatment, totaling 100 *D. suzukii* adults per treatment.

5.3.2 Pure extract of oxymatrine assay

Adults of *D. suzukii* used in this assay were obtained from the laboratory-reared culture in Groningen, the Netherlands, that had been started in 2013 from about 100 individuals, collected in France (GPS coordinates: 43.754059 N, 4.4595 E). All flies had a summer phenotype and were provided with an artificial diet containing agar (10 g), sucrose (15 g), glucose (30 g), corn meal (15 g), wheat germ (10 g), soy flour (10 g), molasses (30 g), yeast (35 g), propionic acid (5 ml), and nipagin 10% (10 ml) and 1 L of distilled water, that served as both a food source and an oviposition medium. The insects were kept under rearing conditions (20 ± 1 °C, 12:12 light:dark photoperiod, 70% RH). The pure extract of oxymatrine (Matrine N-oxide) was manufactured by AdooQ Bioscience (California, United States of America) with 98% purity.

To assess variation in the consumption of different concentrations of oxymatrine, we used the capillary feeder (CAFE) assay (Ja *et al.*, 2007). The experimental unit was composed of a plastic vial (size 25 mm x 90 mm, 35 ml), filled in the bottom with 5 ml of 1.7% agar and closed by a sponge foam containing a capillary tube of 25 µl (Hirschmann minicaps®) in the middle. Three females of 15 days old were placed inside of each vial. The capillary tube was filled with the solution composed of 20% sugar solution + oxymatrine in concentrations of 0% (control), 0.5%, 1.0%, 2.5%, 5.0%, and 7.5%. The voluntary consumption of the solution was quantified at 2 and 24 hours after exposure by measuring how much the volume in the capillaries was reduced. To obtain the actual solution consumption, and to correct for potentially differential evaporation between treatments, we also measured volume reduction in control vials without flies; the volume lost through evaporation was subtracted from the solution consumption by the flies.

To link the consumption of oxymatrine to the mortality of flies, the capillary tube was removed from the vial after 24 hours of exposure, and the flies were kept inside a plastic vial for around 40 hours to assess the female mortality. A completely randomized experimental design was adopted, with 10 replications per treatment, totaling 30 *D. suzukii* females per treatment. The bioassay was performed two times.

5.3.3 Data analysis

The Kaplan-Meier survival plot was used to represent the mortality of *D. suzukii* males and females throughout the package “survival” (Therneau, 2023). Adult mortality data obtained from the bioassay with the commercial product Matrine® were used to calculate insecticide efficiency using the Abbott formula Ma = (Mt - Mc)/(100 - Mc) x 100, where Ma = mortality corrected in the function of the control treatment; Mt = mortality observed in the insecticide treatment and Mc = mortality observed in the control treatment (Abbot, 1925). Adult mortality data were analyzed considering a model with Binomial dispersion parameters. For the model’s selection, the Akaike information criterion and diagnostic graphs were used.

The solution consumption and mortality data were analyzed using the generalized linear mixed-effects model (GLMM) throughout the package lme4, and the assay replication was considered a random effect. In the solution consumption model, the factors of treatment and time of exposure were considered. The boxplot figure was made using the package ggplot2 (Wickham, 2016). All analyses were performed in the R environment considering a 5% significance level (R Core Team, 2021).

5.4 RESULTS

5.4.1 Commercial matrine-based insecticide

The survival curve shows that all the tested doses of the Matrine® induced mortality in both female and male *D. suzukii* (Figure 12). Higher doses resulted in higher mortality. The mortality rate was consistently higher in males than in females. The peak mortality for both males and females occurred at 72 hours, reaching maximally 43% for females and 77% for males. Males exhibited over 48% mortality within the first 24 hours after exposure to doses above 1.0 ml L^{-1} (Figure 12).

The highest *D. suzukii* adult mortality rate (58%) was observed 72 hours after exposure to the maximum dose tested (1.4 ml L^{-1}). This rate significantly differed from the lower doses tested (0.8- and 1.0-ml L^{-1}) (Table 11). Higher doses consistently exhibited elevated mortality rates, from the first assessment at 12 hours, throughout the full 72 hours (Table 11).

Figure 12 - Kaplan-Meier survival curve for *Drosophila suzukii* female (A) and male (B) at 12, 24, 36, 48, 60, and 72 hours after exposure to different doses of Matrine® insecticide.

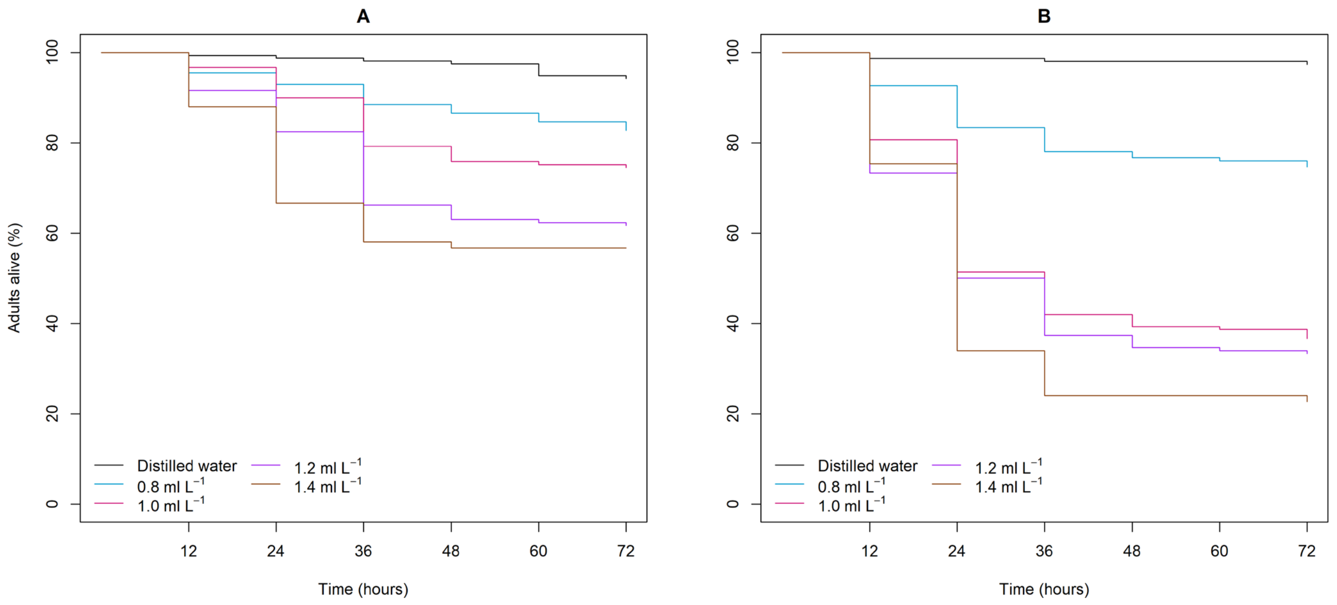


Table 11. Cumulative mortality* of Drosophila suzukii adults (M% ± SE) exposed to different doses of Matrine® insecticide at each exposure time (in hours).

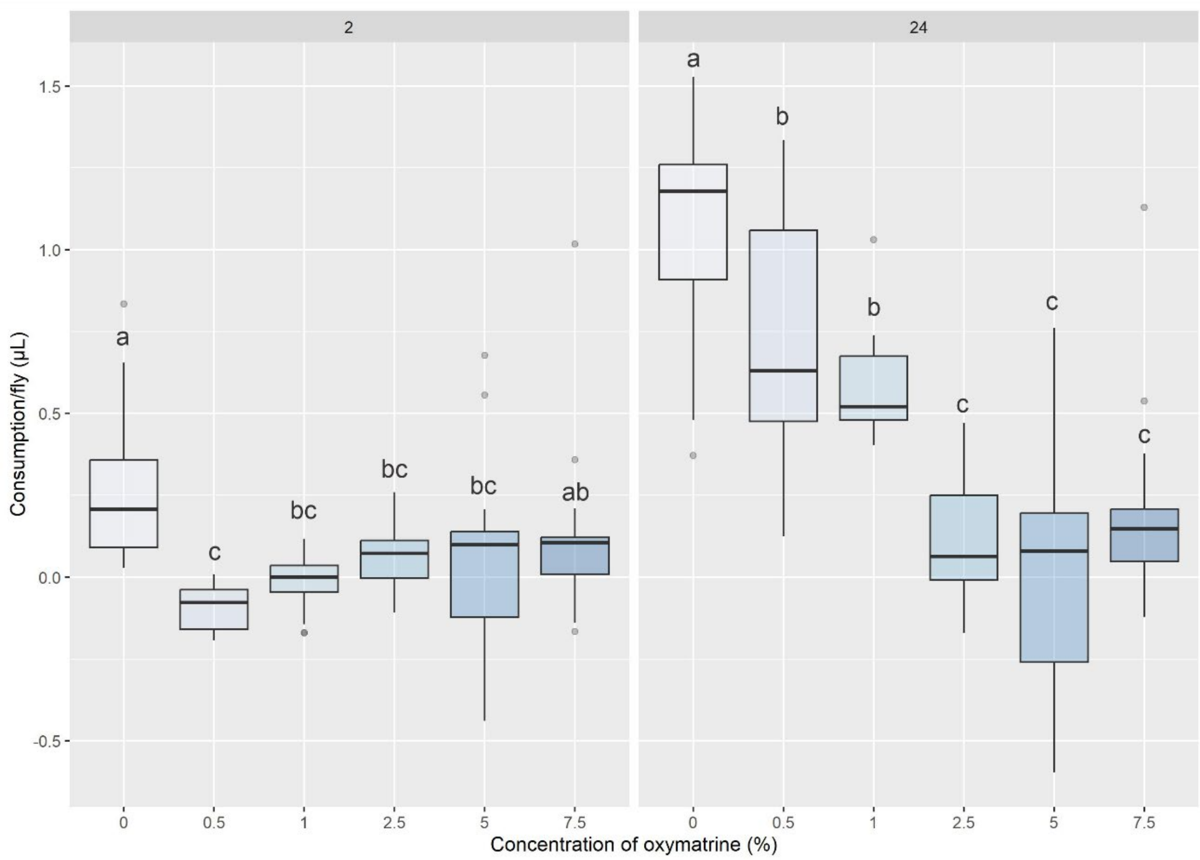
Dose**	**Cumulative Mortality (%) ± SE**					
	12	**24**	**36**	**48**	**60**	**72**
0.8	04.54±01.53b	10.32±01.91c	14.88±0.2.13c	16.22±02.37c	16.44±02.25c	17.16±02.28c
1.0	09.74±01.84ab	28.55±03.53b	38.40±04.14b	41.40±04.01b	40.96±04.14b	42.09±03.99b
1.2	17.00±02.40a	33.58±03.18b	47.45±03.81ab	50.12±03.60ab	49.60±03.86ab	49.56±03.99ab
1.4	16.90±02.88a	49.12±02.98a	58.16±03.12a	58.83±03.17a	58.38±03.21a	58.95±03.19a
p-value	>0.0001	>0.0001	>0.0001	>0.0001	>0.0001	>0.0001

Means followed by the same letter, in the column, do not differ statistically from each other, according to the Tukey test ($p<0.05$). *Corrected by Abbott's formula. ** Dose in ml L^{-1} water.

5.4.2 Pure extract of oxymatrine

Solution consumption of oxymatrine by *D. suzukii* females was observed for all the tested concentrations after 24 hours of exposure (Figure 13). We observed some phago-repellency of oxymatrine because the control (20% of sugar) always showed the highest solution consumption rate (Figure 13). During the first 2 hours of exposure, the consumption was low for all doses, compared to the control, with the least consumption for the lowest concentration. However, after 24 hours, it was the higher concentrations that were the least consumed by *D. suzukii* females (Figure 13). The consumption of the three highest concentrations was hardly increased compared to the 2-hour time point, and for the two lower concentrations, consumption was still lower than for the control.

Figure 13 - Solution consumption of different oxymatrine concentrations by *Drosophila suzukii* females in 2 and 24 hours of exposition in Capillary Feeder (CAFE) assay.



Averages followed by the same letter, within a timepoint, do not differ statistically from each other, according to Tukey's test (p<0.05).

The negative consumption values in CAFE assays can be partially explained by the variability in evaporation, and the effect of oxymatrine concentration on evaporation rate (Table 12). A significant effect of concentration (p-value 0.0004) in the evaporation controls was identified. This means that the concentration of oxymatrine in the solution can influence the evaporation rate. In general, the higher concentrations of matrine also presented the higher evaporation rate in 24 hours (Table 12).

Table 12 - Evaporation (E ± SE) (μL) of the controls for each treatment with different oxymatrine concentrations.

Controls of Treatments	**E ± SE**	
	2 hours	**24 hours**
0%	1.31 ± 0.15 b	8.72 ± 0.19 b
0.5 %	1.62 ± 0.16 ab	8.83 ±0 .29 b
1.0%	1.45 ± 0.15 b	8.49 ± 0.26 b
2.5%	1.42 ± 0.09 b	8.92 ± 0.03 b
5.0%	2.20 ± 0.34 a	10.14 ± 0.46 a
7.5%	1.90 ± 0.26 ab	10.38 ± 0.31 a
p-value	0.0001	<0.0001

Averages followed by the same letter, in the column, do not differ statistically from each other, by Tukey's test (p<0.05).

We observed mortality after 40h exposure for the oxymatrine concentrations of 2.5, 5.0, and 7.5% (Table 13). Among the treatments that caused mortality, the highest concentration (7.5% Oxymatrine) resulted in the highest mortality rate (Table 3). There was no significant correlation between solution consumption in 24 hours and female mortality (Spearman correlation) (Table 13).

Table 13 - Mortality average (M ± SE) of *Drosophila suzukii* females after 40 hours of exposure to different concentrations of oxymatrine and Spearman correlation (SC) between solution consumption and female mortality

Treatments	**M ± SE**	**Spearman Correlation**	
		rho	p-value
0%*	00±00	-	-
0.5 %*	00±00	-	-
1.0%*	00±00	-	-
2.5%	13±20b	-0.1400	0.5609
5.0%	15±23b	-0.0300	0.8930
7.5%	38±33a	0.0100	0.9790
p-value	0.0016		

* The treatments were excluded from the analysis. Averages followed by the same letter, in the column, do not differ statistically from each other, by Tukey's test (p<0.05).

5.5 DISCUSSION

In this study, we aimed to assess the potential of botanical compounds obtained from the medical herb *Sophora flavescens* (matrine and oxymatrine) as insecticides for controlling *D. suzukii*. To investigate this, we conducted two sets of experiments to evaluate the toxicity, one with exposure to a droplet and the other in a capillary feeding (CAFE) assay. The results demonstrated that both substances, matrine and oxymatrine, were toxic to females and caused mortality. In the CAFE assay, we observed that oxymatrine caused *D. suzukii* female mortality through ingestion, but it also showed phago-repellency. The mortality effect of matrine and oxymatrine has been studied in agricultural pests, and ongoing research is exploring their mechanism of action (Ali *et al.*, 2017; Bloomquist *et al.*, 2018; Celiz; Ubaub, 2019; Liu *et al.*, 2008; Zanardi *et al.*, 2015).

All tested doses of the commercial matrine-based product Matrine® caused mortality in adults of *D. suzukii*. This aligns with the findings of Zanardi *et al*. (2015), who also tested the toxicity of a matrine-based (0.36%) formulation to *Diaphorina citri*, *Panonychus citri*, *Sitophilus zeamais*, and *Spodoptera frugiperda* in both laboratory and field assays. The authors reported that the effect of the matrine-based formulation depended on concentration, exposure time, and insect species, where *P. citri* was the most susceptible and *D. citri* the least. Celiz, Ubaub (2019) tested different concentrations of matrine (0.05%, 0.1%, and 0.15%) and observed a mortality effect in *Thrips hawaiiensis.* The 0.1% concentration of matrine resulted in a higher mortality rate than the insecticide abamectin (which was used as a positive control).

In this study, only the higher oxymatrine concentrations (2.5%, 5.0%, and 7.5%) caused mortality in *D. suzukii* at rates ranging from 13% to 38% respectively. This is in concordance with other research results. Using different doses of oxymatrine to control second and fourth larval instars and pupae of *Culex* species in the field, Mohamed *et al*. (2023) established that at least 50% lethality (LC_{50}) was obtained with doses of 5.73, 8.74, and 16.44 ml L^{-1}, corresponding to concentrations levels lower than 1.5% in our assay. Similarly, Gadallah *et al*. (2014) using a commercial product composed of 0.2% of oxymatrine to control second-instar larvae of *Sesamia critica*, observed that the mortality rate increased by increasing the concentration of the commercial product, reaching 100% larval mortality after 12 days at the concentrations of 1.05 and 1.50 ml L^{-1}. This suggests that the life stage of the insect may influence the dose response for oxymatrine.

Besides causing mortality, in this study, the Matrine® insecticide affected insect mobility by reducing their movement after contact with the insecticide droplet. This effect can be related to the mode of action on the nervous system which has been recently investigated. Liu *et al*. (2008) found that matrine and oxymatrine can bind to the nicotinic acetylcholine receptors (nAChRs) in *Periplaneta americana*, acting as antagonists once bound to these receptors. Similarly, Ali *et al*. (2017) reported that the mortality effect observed in *Bemisia tabaci* after exposure to matrine in combination with an entomopathogen, is related to acetylcholine production. The enzyme acetylcholinesterase (AchE), as well as the nervous system, are important target sites for the action of insecticide since they regulate insect movement and behavior (Umar; Aisami, 2020).

Considering that fruit flies like *D. suzukii* are insects that have a high capacity to disperse through flight (Cahenzli; Strack; Daniel, 2018), having an insecticide that reduces female movement could lead to a reduction in oviposition behavior, thus preventing damage to fruits. Bloomquist *et al*. (2018) identified sluggish behavior in larval central nervous systems of *D. melanogaster* due to matrine, reminiscent of GABA (Gamma-Aminobutyric Acid) interference. In *Aphis citricola* juveniles, matrine inhibited HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), which regulates insect juvenile hormones biosynthesis (Xu *et al.*, 2020). Even though toxicology tests on the insecticides Matrine® and oxymatrine on *D. suzukii* were not carried out in this study, it is strongly recommended to find out, as these compounds may act on different sites depending on the life stage of the insect.

In addition to causing mortality, the pure extract of oxymatrine, tested through a CAFE assay, exhibited antifeedant/repellent properties. The observation of low solution consumption at all concentrations, even as low as a concentration of 0.5%, at 2 hours of exposure suggests that oxymatrine is not tasty to *D. suzukii* females. It makes a sugar solution, which typically acts as a phagostimulant, unpalatable. These findings align with the work of Celiz, Ubaub (2019) who observed a reduction in damage by *Thrips hawaiiensis* when banana fruit was treated with a matrine-based product, indicating antifeedant properties. Similarly, Kordestani *et al*. (2022) reported the antifeedant and repellent effects of matrine, altering the prey’s taste and reducing the consumption by the predator *Orius laevigatus.* Zanardi *et al*. (2015) in their assessment of a matrine-based biopesticide, also identified antifeedant effects on *D. citri,* a reduction in the number of eggs laid by *P. citri* females, and an impact on the larval development of *S. frugiperda*.

When matrine undergoes oxidation to become oxymatrine, and its activity towards nAChRs is reduced (Liu *et al.*, 2008). This transformation may be a contributing factor to the differences observed in this study. The commercial matrine-based product Matrine®, corresponding to 0.2% oxymatrine dose, was more harmful to *D. suzukii* with a mortality rate reaching almost 60%, than the pure extract of oxymatrine at different concentrations ranging from 0.5% to 7.0%, reaching mortality rates up to 38%. Interestingly, even at lower doses (1.0 ml L^{-1}), the insecticide matrine-based Matrine® resulted in a higher mortality rate (58%) in females compared to the pure extract of oxymatrine at higher concentrations, where 7.5% caused up to 38%, for the same period of 40 hours after exposure.

Biological parameters of *D. suzukii* including sex, age, and genetic diversity, could contribute to the variations observed in the study results. Blouquy *et al*. (2021) identified that sex and age can lead to different results. In this study, flies were either five-day-olds (Matrine®) or fifteen-day-olds (oxymatrine), and the two experiments were conducted with two different *D. suzukii* populations. Comparing sexes within the same assay (Matrine®) in this study, it was evident that males required lower doses to achieve the same mortality rate as females. Although it is the females that cause the damage, targeting the males is also important. In bioassays with the insecticide lambda-cyhalothrin, the fertility of *D. suzukii* female was not affected by exposure to the insecticide after mating (Krüger *et al.*, 2021). Thus, avoiding mating through inducing male mortality may contribute to the reduction of the *D. suzukii* population. Blouquy *et al*. (2021) also tested insecticide susceptibility in two populations of *D. suzukii*, finding no differences. However, the populations tested by the authors were a low genetic diversity population (SF-IsoA) generated from the Ste-Foy population. In our bioassays, two distinct *D. suzukii* populations from different countries and continents were utilized. The genetic background and diversity of both populations might also cause differences in mortality rates when they differ in resistance or tolerance for the tested compounds (Fraimout *et al.*, 2017). Moreover, the botanical compounds we used had different active compounds and came from different manufacturers. All this implies that a direct and quantitative comparison between the results for the two experiments is not warranted. However, the combined experiments both indicated that there is potential for developing the botanical compounds obtained from the medical herb *Sophora flavescens* (matrine and oxymatrine) as insecticides for controlling *D. suzukii*.

5.6 CONCLUSION

Although the commercial product Matrine® demonstrated a mortality effect on *D. suzukii*, the mortality rate was lower than 60% even at the highest dose tested (1.4 ml L^{-1}). In Brazil, this product is available in the market. However, the Ministry of Agriculture, Livestock, and Supply (MAPA) requires that mortality rate is over 85% in a laboratory test for new products to be registered. Given the potential demonstrated in this study, it is recommended that future research focuses on determining the lethal dose that is necessary for *D. suzukii*. The identification of mortality effects through oxymatrine ingestion in CAFE assay showed that this botanical compound could be explored as the killing agent in toxic bait formulations against *D. suzukii*. The toxic bait would need to overcome the phago-repelling effects that the taste of oxymatrine seems to have. Furthermore, when it can be made to be specifically attractive to the target insect, this would reduce the amount of insecticide applied in the field and the non-target effects it could cause.

Matrine-based insecticide (Matrine®) is a green product option, as it comes from a plant extract. It could serve as an alternative to spinosyn insecticide, particularly for organic fruit production systems, which is the main insecticide currently used to manage *D. suzukii* in the field. It is important to have more than one insecticide molecule to be used in integrated pest management, to avoid the evolution of insect resistance. Furthermore, matrine/oxymatrine presents some benefits such as low persistence in the field, minimal impact on the reproduction of the predator *O. laevigatus* and synergistic effects when combined with the entomopathogenic fungus *Lecanicillium muscarium* (Ali *et al.*, 2017; Kordestani *et al.*, 2022; Zanardi *et al.*, 2015). This underscores the scope and need for further research and field studies for *D. suzukii* control.

6 DO HIGH DYNAMIZED DILUTIONS CHANGE THE BIOECOLOGY ASPECTS OF SPOTTED-WING DROSOPHILA?

6.1 ABSTRACT

The use of high dynamized dilution (HDD) to sustainably improve crop production has been well reported. However, the use of HDD to control insects in crop protection remains an understudied area. Thus, a study was carried out to verify the activity of HDD on *Drosophila suzukii* bioecology aspects using two different methodologies. The high dynamized dilutions tested were a nosode of *D. suzukii* 19CH, *Bufo rana* 19CH, *Sepia succus* 19CH, *Hyoscyamus niger* 19CH, and *Lilium tigrinum* 19CH. Distilled water and a 1.10^{-19} dilution of lactose were used as controls. First, the treatments were offered to *D. suzukii* females in a 20% sugar solution in the Capillary Feeder (CAFE) assay for 24 hours. The consumption of solutions was evaluated at 2, 18, and 24 hours. The effects on reproduction were assessed by providing the flies with an oviposition substrate for 24h and counting egg-laying and emerged adults. To evaluate the effect of HDD on reproduction after both the parental flies and juveniles are exposed to HDD, adults *D. suzukii* were transferred to a plastic vial containing 5 ml of medium diet + 50 µL of HDD treatment for 24 hours for oviposition. Afterward, the juveniles were kept on the HDD-treated diet until adult’s emergence. When the F1 progeny was 15 days old, the number of eggs laid for 24 hours was assessed. The high dynamized dilutions stimulated *D. suzukii* consumption and led to increased reproduction in adults. Reproduction was also increased for the F1 progeny that had developed on HDD diet as juveniles. For pest management, the tested HDDs are not suitable, as they enhanced the reproduction of the pest insect.
Keywords: *Drosophila suzukii*, agroecology, homeopathy, sustainability.

6.2 INTRODUCTION

The use of high dynamized dilution (HDD) has been proposed as an eco-friendly and cost-effective strategy to manage pests and diseases in agriculture (Giesel; Boff; Boff, 2012; Verdi *et al.*, 2020). The HDD is characterized by preparations produced through the method of sequential dilutions and succussions, also known as dynamization (Sen *et al.*, 2018). With repeated dilutions and succussions, HDD reaches a level of potency where its particles become infinitesimal and undetectable (Boff; Verdi; Faedo, 2021). As it is pointed out by Moreno (2017), HDD causes no toxicity to plants, animals, humans, and the environment. Moreover, compared to chemical fertilizers/pesticides, the amount of "mother tincture" needed to produce the final preparation of HDD is so low as well as the amount applied, that this technique can be considered low-cost and accessible to all farmers (Boff; Verdi; Faedo, 2021). In agriculture, the first studies made by Kolisko, Kolisko (1978) tested the effect of HDD preparations on plants. Since then, studies with HDD have shown that it can stimulate plants and animals' health to pass over adverse environmental conditions or pest and disease attacks (Modolon *et al.*, 2012).

The use of insects as a model to investigate the effects of HDD in crop protection remains an understudied area in the global context. Nonetheless, select studies involving insects like the velvetbean caterpillar (*Anticarsia gemmatalis*), the fall armyworm (*Spodoptera frugiperda*), and the leafworm kale (*Ascia monuste orseis*) have shed light on the potential for HDD to alter caterpillar feeding behaviors, leading to a reduction in adult emergence (Mapeli *et al.*, 2015; Modolon *et al.*, 2017; Silva *et al.*, 2023). Reduction of damages in tomato fruit by small borer (*Neoleucinodes elegantalis*) after the plants received applications of HDD as well as reduction in larvae infestation in peach fruits by South American fruit fly (*Anastrepha fraterculus*) after spraying HDD in orchards, were already reported (Modolon *et al.*, 2012; Rupp *et al.*, 2012). Additionally, the silkworm (*Bombyx mori*) fed with plant leaves treated with HDD increased silk production (Avhad; Pagare; Hiware, 2015).

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) known as spotted-wing drosophila (SWD) is an invasive pest native from Asia and already worldwide spread (Garcia, 2020). Since it was first registered in Europe and North America in 2008, SWD has been identified as causing large economic losses to fresh fruit production (Asplen *et al.*, 2015; Farnsworth *et al.*, 2017). The geographic distribution of SWD includes South America and Africa (Boughdad *et al.*, 2021; Deprá *et al.*, 2014). This is possible due to its plasticity to adapt to different climate conditions, having a wide host range, and short lifecycle which also gives *D. suzukii* the status of a global pest (Poyet *et al.*, 2015; Tochen *et al.*, 2014). Additionally, *D. suzukii* has the potential to become introduced to other important fruit production regions such as Australia and New Zealand, where it has not been recorded yet (Ørsted; Ørsted, 2019).

The *D. suzukii* females exhibit a distinctive behavior by laying their eggs inside the epicarp of fruits. This peculiarity, facilitated by their serrated ovipositor, inevitably results in punctures that damage the fruit, rendering it unmarketable (Garcia, 2020). However, the main fruit damage is caused by the larvae that feed inside the fruits. Management of *D. suzukii* is challenging, not only because of its wide host range and adaptability to different environments, but it is also difficult to target the immature stages (egg, larvae, and pupae) once they occur inside the fruit (Funes *et al.*, 2018). Currently, the control of *D. suzukii* relies on insecticide sprays, such as organophosphates, pyrethroids, and spinosyns, to primarily target the adult population (Diepenbrock; Hardin; Burrack, 2017). Normally insecticides have broad-spectrum lethality, killing a wide range of insects, and potentially resulting in a negative effect on natural enemies, human health, and the environment (Chagnon *et al.*, 2015). Alternative methods as eco-friendly and cost-effective strategies are urgently needed to reduce insecticide use and, develop an integrated control strategy for effective management to keep low SWD infestations (Tait *et al.*, 2021).

As a first step to evaluate the potential of HDD for the pest management of *D. suzukii*, we need to characterize how various HDDs can affect the biology of the target insect, since it can be variable in insects, depending on the insects' bioecology. Here, we evaluated the effect of high dynamized dilution on *D. suzukii* on reproduction. We measured the consumption of *D. suzukii* females of solutions containing high dynamized dilutions and measured its effects on reproduction both in parents and in the F1 progeny.

6.3 MATERIAL AND METHODS

6.3.1 Insect culturing

The studies were performed in bioassays under laboratory conditions at the Groningen Institute for Evolutionary Life Sciences of the University of Groningen, The Netherlands. Adults of *D. suzukii* were obtained from the laboratory-reared culture that had been started in 2013 from about 100 individuals, collected in France (GPS coordinates: 43.754059 N, 4.4595 E). All the flies in the study exhibited a summer morphotype and were provided with an artificial diet consisting of the following components: agar 10 g L^{-1}, sucrose 15 g L^{-1}, glucose 30 g L^{-1}, corn meal 15 g L^{-1}, wheat germ 10 g L^{-1}, soy flour 10 g L^{-1}, molasses 30 g L^{-1}, yeast 35 g L^{-1}, propionic acid 5 ml L^{-1}, nipagin 10% 10 ml L^{-1}, both as a food source and an oviposition medium. These insects were maintained under controlled rearing conditions, which included a temperature of 20 ± 1 °C, a 12-hour light and 12-hour dark photoperiod, and a relative humidity of 70%. The bioassays were also conducted in a controlled environment within a climatized room, maintaining conditions at 20 ± 1 °C, with a 12-hour light and 12-hour dark photoperiod, and a relative humidity of 70%.

6.3.2 High dynamized dilutions

The high dynamized dilutions tested were (a) *D. suzukii* Nosode 19CH (nineteenth centesimal Hahnemannian), (b) *Bufo rana* 19CH, (c) *Sepia succus* 19CH, (d) *Hyoscyamus niger* 19CH and (e) *Lilium tigrinum* 19CH. The choice of these treatments, *Bufo rana*, *Sepia succus*, *Hyosciamus niger,* and *Lilium tigrinum* was based on analogies with Materia Medica (Vijnovsky, 1980) while considering their potential impact on the reproductive system of female flies. The nosode treatment was prepared by triturating adult *D. suzukii* specimens with lactose, following the methodology outlined in the Brazilian Homeopathic Pharmacopeia (Brasil, 2011). As control treatments, we used distilled water as the official standard in plant-pest experimentation, and lactose 1.10^{-19} as a carrier control to nosode diluted in distilled water (Wyss *et al.*, 2010). The control lactose was obtained from De Hahnemann Apotheek BV, The Netherlands.

The bioassays were conducted following a double-blind procedure, ensuring that neither the researcher nor the technician were aware of the specific treatments being administered, as they were identified only by codes. The treatment codes remained undisclosed until the data processing phase; a practice implemented to eliminate the potential for subjective influences (placebo effects). The treatments were revealed and associated with their respective codes only after the data analysis was completed.

6.3.3 Consumption and parental reproduction

The capillary feeder (CAFE) assay was performed to assess variation in the consumption of the HDD treatments (Ja *et al.*, 2007). The HDD and control treatments were offered to *D. suzukii* females, mixing them 1:10 v/v in a 20% sugar solution as an energy source. The experimental unit was composed of a plastic vial (size 25 mm x 90 mm, 35 ml), filled in the bottom with 5 ml of 1.7% agar and closed by a sponge foam containing a capillary tube of 25 µl (Hirschmann minicaps®) in the middle. Into each plastic vial, three 15-day-old females were released, and the voluntary consumption from the capillary tube was evaluated at 2, 18, and 24 hours after exposure. To obtain approximately the real consumption volume, and to correct for potentially differential evaporation between treatments, the evaporation measured in control vials without flies was subtracted from the consumption by the flies.

After 24 hours of exposure to the treatments, the females were transferred to a new plastic vial for 24 hours to lay eggs, and this was repeated with a transfer to new vials for another 24h hours. The vials for egg laying for the first 24h contained 5 ml of a medium diet, containing yeast 26 g L^{-1}, agar 17 g L^{-1}, sugar 54 g L^{-1}, and nipagin 10% 17 ml L^{-1}. These vials with the medium diet and eggs were then maintained under the same controlled conditions as the *Drosophila* culture (20 ± 1 °C, 12:12 light:dark photoperiod, 70% RH) until adult emergence. Adult emergence was evaluated for 7 days from the first adult emergence onwards. The vials for egg laying for the second 24h contained 5 ml of 1.7% agar. After this second period of 24h, the number of eggs was counted. The experimental design was completely randomized with respect to HDDs assigned to vials, with 10 replications per treatment, performed three times, involving a total of 210 *D. suzukii* females each time.

6.3.4 Reproduction after parental and juvenile exposure to HDD

The bioassay was performed using 15-day-old *D. suzukii* adults. Each replicate unit consisted of three *D. suzukii* females and one male, in a plastic vial (size 25 mm x 90 mm, 35 ml) filled with 5 ml of medium diet (yeast 26 g L^{-1}, agar 17 g L^{-1}, sugar 54 g L^{-1} and nipagin 10% 17 ml L^{-1}). Using a pipet, 50 µl of each HDD treatment was mixed 1:10 v/v with distilled water and applied on top of the diet in the vial, 2 hours before the flies were released inside the vial. The purpose of this step was to allow the medium diet to absorb the HDD. After the adult flies were released into the vials containing the medium diet with the HDD treatment, they were kept in these vials for 24 hours to enable the *D. suzukii* females to lay eggs. After this incubation period, the adult flies were removed from the vials. The vials, now containing medium diets with HDD treatment and infested with *D. suzukii* eggs, were subsequently maintained under the same controlled environmental conditions as the *Drosophila* culture. The development from egg to adult was monitored in these vials.

To evaluate the impact of HDD preparations during juvenile development on subsequent reproduction after emergence, we recorded and collected the adults that emerged from each treatment for 7 days from the first adult emergence onwards. Subsequently, all the emerged adults from one vial (i.e., the "F1 progeny" from here on onwards) were transferred to a new plastic container with a capacity of around 180 ml (6 oz), and these containers contained 40 ml of the medium diet. The F1 progeny were maintained in these containers for approximately 15 days, to reach reproductive age and for mating. Then, two females and one male of each container with F1 progeny were released in a plastic vial (size 25 mm x 90 mm, 35 ml) containing 5 ml of 1.7% agar for 24 hours. The adults were then discarded, and the number of eggs was counted to evaluate the reproduction of the F1 progeny. The experimental design was completely randomized with respect to flies assigned to HDD treatments, with 10 replications per treatment, performed twice, using a total of 280 *D. suzukii* adults as parents and 210 *D. suzukii* from the F1 progeny each time.

6.3.5 Data analysis

The consumption and reproduction data were analyzed using generalized linear mixed-effects models (GLMM), using the package lme4 (Bates *et al.*, 2015). The replication of each experiment was specified as a random effect. In the consumption model, the factors of treatment and time of exposure were considered, and a Gaussian distribution was specified. Adult emergence and reproduction data were analyzed with treatment as a fixed effect and specifying the Poisson distribution. Spearman correlation was performed between the consumption and reproduction measurements. The analysis of the number of eggs, laid by females after consuming the treatments in the CAFE assay, used the Generalized Additive Models for Location Scale and Shape (GAMLSS) (Rigby; Stasinopoulos; Lane, 2005). The best model (Negative Binomial type II) was chosen using the function 'chooseDist'. For all the model selection, the

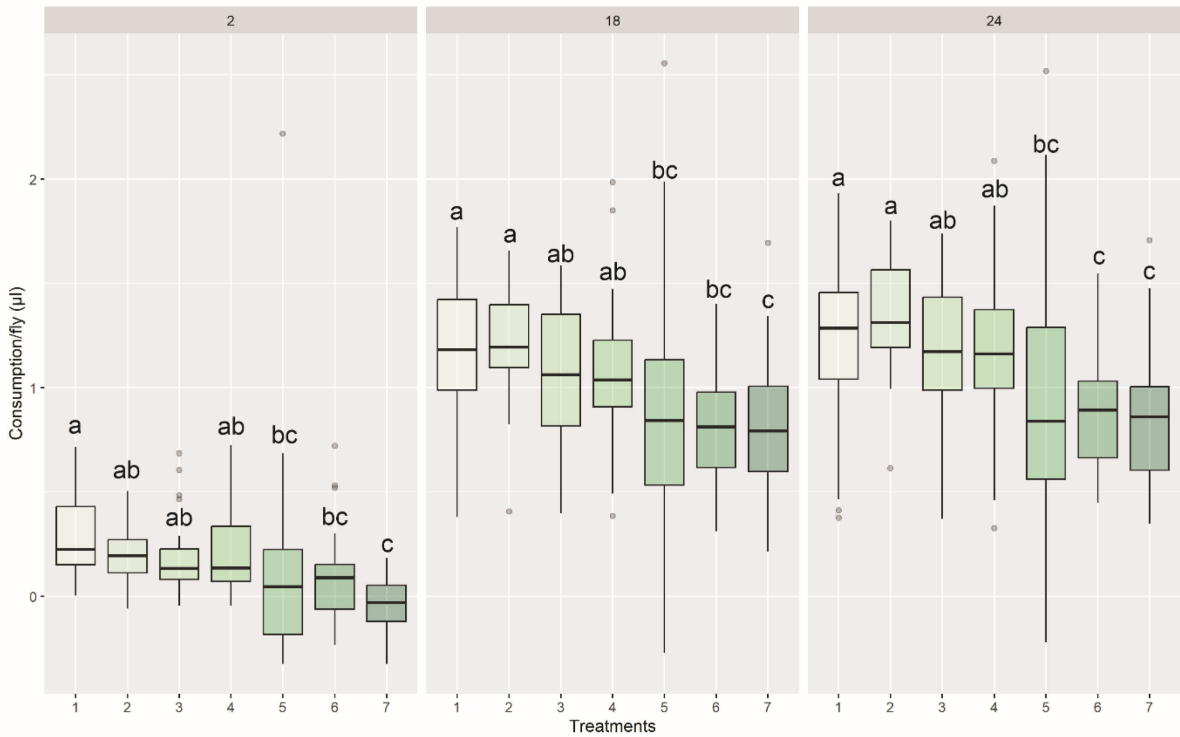
Akaike information criterion and diagnostic graphs were used. The consumption figure was made using the package ggplot2 (Wickham, 2016). All analyses were performed in the R environment considering a 5% significance level (R Core Team, 2021).

6.4 RESULTS

6.4.1 Consumption and parental reproduction

The *D. suzukii* females' consumption in the CAFE assay (Figure 14) significantly increased over time during the exposure period (*p*-value = 0.0200). The *D. suzukii* nosode 19CH treatment was the most consumed for all the periods of exposure, with consumption of 1.23 µl per fly over 24 hours. In contrast, the distilled water control exhibited the lowest consumption, at 0.84 µl per fly over 24 hours (Figure 14).

Figure 14 - Consumption of high dynamized dilution by *Drosophila suzukii* females after 2, 18, and 24 hours of exposure in the Capillary Feeder (CAFE) Assay.



Treatments: 1) *D. suzukii* nosode19CH; 2) *Hyoscyamus niger* 19CH; 3) *Lilium tigrinum* 19CH; 4) *Sepia succus* 19CH; 5) *Bufo rana* 19CH; 6) Lactose 1.10^{-19}; and 7) Distilled water. Averages followed by the same letter, in the time, do not differ statistically from each other, by Tukey's test (p<0.05).

The negative consumption values in some treatments indicate that the reduction in volume in the evaporation controls was higher than the evaporation and consumption by the flies combined. Especially during the first 2h, these negative consumption values suggest that the flies did not feed until the first evaluation took place (Figure 14).

At the final evaluation, after 24 hours of exposure, the treatment *Hyosciamus niger* 19CH exhibited the highest consumption (1.35 µl/fly) whereas the lowest consumption rate was recorded for the two control treatments, lactose 1.10^{-19} (0.88 µl/fly) and distilled water (0.84 µl/fly). All HDD treatments, except for *Bufo rana* 19CH, were not significantly different from the higher consumption of the *Hyosciamus niger* 19CH treatment, and the *Bufo rana* 19CH HDD was not significantly different from the two control treatments (Figure 14).

Next, we evaluated whether the consumption of HDD affected reproduction. We first assessed a relation between the consumption of HDD and the number of offspring that were produced, counting the adult emergence from eggs that were laid during the first 24 hours after the consumption of HDD treatments or the controls. This showed that the number of offspring produced (measured as emerged adults) was significantly higher after consumption of *Hyosciamus niger* 19CH than in both controls (Table 14). The offspring produced was also significantly higher after *Bufo rana* 19CH consumption, compared to the distilled water control (Table 14). All other pairwise comparisons showed some overlap with other treatments or controls (Table 14).

Table 14 - Reproduction of *Drosophila suzukii* after consumption of high dynamized dilutions in a capillary feeding (CAFE) assay. The first column summarizes the number of progenies that were produced from eggs laid in the first 24 hours after the CAFE assay, as measured by counting emerged adults (Adult Emergence ± SE). The second column summarizes the number of eggs that were laid in the second 24h after the CAFE assay (Egg count ± SE)

Treatment	Adult emergence ±SE	Egg count ±SE*
***D. suzukii* nosode 19CH**	9.73±1.72 abc	8.26±1.62 a
***Hyosciamus niger* 19CH**	11.26±1.82 a	5.90±1.73 ab
***Lilium tigrinum* 19CH**	8.30±1.50 bc	5.93±1.50 ab
***Sepia succus* 19CH**	8.90±1.56 abc	8.66±2.75 ab
***Bufo rana* 19CH**	10.53±1.67 ab	10.60±2.59 ab
Lactose 1.10^{-19} (control)	8.80±1.58 bc	5.96±2.16 b
Distilled water (control)	7.63±1.49 c	9.40±2.14 a
p-value	<0.0001	0.0377

Averages followed by the same letter, in the column, do not differ statistically from each other by Tukey's test (p<0.05). *The analysis was performed using gamlss.

Secondly, we transferred the females to vials containing 1.7% agar and counted the number of eggs that were laid by females during the next 24 hours. When examining the number of eggs across treatments, these were significantly different between treatments (Table 14). This was partly showing a significant difference between the two control treatments. Additionally, the egg count after exposure to *D. suzukii* nosode 19CH was higher than for females exposed to the Lactose control.

Table 15 - Spearman correlation analyses between the consumption (C) of high dynamized dilution (HDD) treatments and the number of offspring produced - as measured by counting adult emergence (A) and the number of eggs laid (E). The consumption was measured in *Drosophila suzukii* females in a Capillary Feeder (CAFE) assay, offering different HDD treatments and controls. For each treatment, the consumption after 2, 18, and 24 hours was correlated to the number of offspring produced in the first 24 hours after the CAFE assay, and the number of eggs laid in the second 24 hours after the CAFE assay.

Treatment	**C x A**		**C x E**	
	rho	**p-value**	**rho**	**p-value**
	2 hours			
***D. suzukii* nosode 19CH**	0.31	0.0919	0.31	0.0969
***Hyosciamus niger* 19CH**	0.20	0.2994	0.06	0.7690
***Lilium tigrinum* 19CH**	0.15	0.4425	0.41	**0.0249**
***Sepia succus* 19CH**	-0.18	0.3412	-0.08	0.6690
***Bufo rana* 19CH**	-0.31	0.0911	0.20	0.2972
Lactose 1.10^{-19} (control)	-0.16	0.3891	0.14	0.4580
Distilled water (control)	-0.30	0.1066	0.18	0.3441
	18 hours			
***D. suzukii* nosode 19CH**	-0.04	0.8236	0.03	0.8748
***Hyosciamus niger* 19CH**	-0.09	0.6449	0.02	0.9087
***Lilium tigrinum* 19CH**	0.24	0.1951	-0.09	0.6278
***Sepia succus* 19CH**	-0.13	0.4916	-0.03	0.8818
***Bufo rana* 19CH**	-0.37	**0.0442**	0.24	0.1922
Lactose 1.10^{-19} (control)	-0.27	0.1522	0.26	0.1721
Distilled water (control)	-0.31	0.0975	0.09	0.6194
	24 hours			
***D. suzukii* nosode 19CH**	-0.14	0.4680	0.04	0.8401
***Hyosciamus niger* 19CH**	-0.14	0.4602	0.08	0.6609
***Lilium tigrinum* 19CH**	0.19	0.3110	-0.06	0.7379
***Sepia succus* 19CH**	-0.20	0.3003	0.01	0.9684
***Bufo rana* 19CH**	-0.37	**0.0454**	0.26	0.1580
Lactose 1.10^{-19} (control)	-0.80	0.6746	0.24	0.2006
Distilled water (control)	-0.21	0.2739	0.07	0.7207

Correlating consumption with reproduction, significant correlations were found for the consumption of HDD *Bufo rana* 19CH and the number of offspring produced, both for their consumption after 18h and 24h (Table 15). These correlations were negative, which means that the less the flies consumed, the greater the emergence of their progeny was. There was also one significant positive correlation between consumption and reproduction: The consumption of *Lilium tigrinum* 19CH after 2 hours was positively correlated with the number of eggs laid (Table 15).

6.4.2 Reproduction after parental and juvenile exposure to high dynamized dilutions

Parental flies were offered an egg-laying substrate containing HDD for 24 hours, and the juveniles were then kept on these substrates to complete their development. We compared the number of offspring that were produced on these substrates (with different HDD treatments) by the parental generation, as well as the reproduction of the F1 progeny.

Table 16 - *Drosophila suzukii* offspring production (Adult emergence ± SE) from eggs that developed in a diet containing the HDD treatments and eggs laid during 24h by 15-day-old F1 progeny (Egg count ± SE) that had developed in a diet containing the HDD treatments. Spearman correlations (SC) between Adults and Eggs for each treatment

Treatment	Adult emergence ±SE	Egg count ±SE	SC	
			rho	p-value
D. suzukii **nosode 19CH**	36.60±2.85 a	16.05±3.89 a	0.33	0.1614
Hyosciamus niger **19CH**	32.20±2.67 ab	8.55±2.08 b	0.04	0.8644
Lilium tigrinum **19CH**	23.25±4.12 cd	4.75±1.44 d	0.06	0.8174
Sepia succus **19CH**	27.65±3.45 abc	13.95±4.13 a	-0.08	0.7264
Bufo rana **19CH**	19.95±2.91 d	5.80±1.92 cd	-0.27	0.2509
Lactose 1.10^{-19} (control)	23.20±2.59 cd	4.65±1.50 d	-0.11	0.6435
Distilled water (control)	22.40±2.84 d	7.50±2.71 bc	0.09	0.7075
p-value	<0.001	<0.001	-	-

All the HHD treatments, except *Bufo rana* 19 CH and *Lilium tigrinum* 19CH showed increased adult emergences, compared to the distilled water control (Table 16). Also, the *D. suzukii* nosode 19CH showed increased adult emergence compared to its Lactose 1.10^{-19} control. When comparing the number of eggs laid by the females that originated from the larvae that developed in the diet with HDD, i.e. the F1 progeny, we find an increased egg production for two of the HDDs, *D. suzukii* nosode 19CH and *Sepia succus* 19CH (Table 16). The other HDDs did not differ significantly from the distilled water control treatment. We did not find any significant correlations between the emergence of F1 progeny, and the numbers of eggs laid for any of the HDD treatments or controls ($p > 0.05$) (Table 16).

6.5 DISCUSSION

In this study, we investigated the effects of HDD on *D. suzukii* reproduction, as a first step to evaluate the potential of these HDDs for pest management. We employed two distinct methodologies, one in which we assessed the consumption rate, using CAFE assay, and then evaluated egg laying and offspring production afterwards. The second methodology was by letting females oviposit in substrates treated with HDD and then measuring the number of adults that emerged and the egg laying by the F1 progeny. Both methodologies showed the effects of HDD on *D. suzukii* reproduction. In several but not all HDD treatments, the offspring or egg laying of parents was increased compared to the controls. Also, the egg laying of F1 progeny showed a significant increase for some HDD treatments compared to the controls. The double-blind execution of these experiments excludes the possibility that these findings can be interpreted as a placebo and reinforces the statement made by Giesel *et al*. (2012) that HDD can affect insect behavior and biology. For pest management, however, it seems that the tested HDD would not be suitable, as they enhanced the reproduction of the pest insect.

In general, HDD stimulated food consumption by *D. suzukii* flies and increased female reproduction. Among the HDD treatments, *D. suzukii* nosode 19CH, *Hyoscyamus niger* 19CH, *Lilium tigrinum* 19CH, and *Sepia succus* 19CH exhibited higher consumption levels when compared to the control groups. However, only the treatment *Hyosciamus niger* 19CH showed a significant difference from the controls in terms of increased subsequent offspring production. In contrast, the HDD *Bufo rana* 19CH, did not show an increased consumption rate, yet the offspring production was increased compared to the distilled water control. The Spearman correlation analyses reflected a similar pattern, where the *Bufo rana* 19CH treatment showed a negative correlation between consumption and offspring production. These stimulatory findings are similar to results reported by Mapeli *et al*. (2015) and da Silva *et al*. (2023) for a range of different HDDs, showing that HDD increased leaf consumption by *Anticarsia gemmatalis* fed with soybean leaves treated with *Calcaria carbonica* 4CH, *Carbo* *vegetabilis* 4CH, *Staphysagria* 4CH and nosode of *Anticarsia gemmatalis* 12CH, and by *Ascia monuste orseis* fed with kale leaves treated with *Sulphur* 12CH. In contrast, Modolon *et al*. (2012) applied *Sulphor* 12CH to tomato plants and observed a reduction in the number of fruits damaged by small borers (*Neoleucinodes elegantalis*). In such a case, an HDD could become part of an integrated pest management approach.

The primary focus of this study was to investigate whether HDD treatment could reduce the reproductive output of the target pest, *D. suzukii*. This was not the case for any of the HDDs we tested. In the context of pest management, a reduction in oviposition would be significant, as it can lead to a reduction in the number or size of new generations. Other case studies were successful in reducing reproduction in a pest insect. For example, Deboni *et al*. (2017) observed that the HDD *A. obtectus* nosode 30CH, *Taraxacum officinale* 30CH, and *Chenopodium ambrosioides* 30CH applied in grain beans did not repel the weevil *Acanthoscelides obtectus*, but reduced the progeny emergence. Additionally, da Silva *et al*. (2023) found that an HDD had an impact on food conversion and caterpillar body length; in this case, however, it did not significantly affect adult emergence, percentage of emerged eggs, or development period. Mapeli *et al*. (2015) also identified lower levels of food conversion in *Ascia monuste orseis* fed with kale leaves treated with HDD and additionally showed lower levels of adult emergence. They stated that the caterpillars fed with kale leaves treated with *Phosphorus* 5CH, *Ruta* 5CH, and *Sulfur* 12CH had developed into adults with shorter wing lengths when compared to the control. In our study, none of the diets containing HDDs resulted in reduced offspring production, while several of the HDDs actually showed significantly increased offspring production. The results of both experiments lend support to the hypothesis that HDDs can enhance the reproduction of *D. suzukii*, regardless of the method of exposure to HDD.

Through what mechanisms the HDD treatment was leading to differences in reproductive output is unknown. From the way we conducted the experiments, we cannot deduce whether the increased offspring production with HDD treatment, as measured from adult emergence, is due to higher fecundity, egg-laying decisions of the females, higher juvenile survival of the offspring, or a combination of those. The egg counts that we conducted in both assays could also reflect effects on either fecundity or egg-laying decisions of the females, or both. The medium that we provided for the offspring production did not allow for making reliable egg counts, and the medium for egg count assays was not suitable for rearing the eggs until adulthood. Furthermore, it is also unknown exactly how HDD treatments may affect the behavior or physiology of insects. Previous research has shown that the application of HDD directly on insects can influence their behavior and activity levels. For example, in the case of leaf-cutting ants by Giesel *et al*. (2012), spraying the HDD directly along the foraging trail, led to a reduced activity after multiple applications. In other studies, it was suggested that HDD may alter the interaction between plants and insects, for example by altering the nutritional content of plants and affecting insect behavior (Mapeli *et al.*, 2015; Silva *et al.*, 2023). Further experiments would be needed to assess choice behavior and physiological changes, as well as the effects on survival of the juvenile stages. Our results were a first step to assess whether there was any detectable effect of HDD on reproduction. Now that we find those for some, but not all, HDD treatments, follow-up research would be needed to tease apart how HDD is influencing reproduction in *D. suzukii*.

Considering that we want to assess whether there is potential for developing HDD as a pest management strategy against *D. suzukii*, we evaluated how HDD affected the flies' behavior. Our experiments were conducted with an artificial diet and sugar solution, which excluded the possibility of interactions between plants and insects. In sugar solutions, we saw an increased consumption in the CAFE assay for solutions containing most, but not all, HDD. This indicates that the tested HDD did not have a repellent effect for feeding. Also, for adults' emergence from artificial substrates with HDD, adults' emergence was either similar or higher than in the control treatments. Thus, the HDD is not a repellent for oviposition either. If anything, reproduction was boosted by HDD. This severely limits the potential of the tested HDD for pest management control.

The duration of required exposure to HDD and for how long any effects persist in insects can vary. We exposed adult flies for up to 24 hours to the HDD for consumption, and measured changes in offspring production or egg laying. This time of exposure was sufficient to observe (future) effects on offspring production during the first 24h, but not on egg laying in the following 24h, as evidenced by the results. The Spearman correlation analysis for consumption and either offspring production or egg laying showed at best weak evidence for any correlation between the amount of consumption and reproduction. Also, when the HDD was applied to the substrate, 24h was sufficient to induce differences in offspring production. This suggests that the effect of this HDD on adults may be short-lived in *D. suzukii*. For juveniles that had spent their entire developmental period on substrates with HDD, the effects were more persistent. We observed differences in offspring production and egg-laying behavior in these F1 progeny when they were 15-day-old adult flies. Also in the earlier mentioned study by Giesel *et al*. (2012), the activity reduction of the leaf-cutting ants treated with a nosode lasted for more than 20 days. Deboni *et al*. (2017) noticed a gradual decrease in HDD effects over time in an assay with *A. obtectus*, with the effects lasting for over 200 hours. This underscores the importance of evaluating HDD effects on a specific insect target, as the duration and magnitude of these effects can vary from one species to another.

Another point about bioassay using HDD that is important to discuss is how the HDD is mixed with other ingredients and its final concentration. For the consumption assays, the HDD was mixed 1:10 v/v with a 20% sugar solution. This resulted in significant effects on *D. suzukii* consumption and reproduction. Also for another HDD, *Iodum* 200CH, Almadani, Hiware (2020), found that concentrations as low as 0.5% showed 99% mortality in the 5th larval instar of *Galleria mellonella* L. Additionally, in the other studies already mentioned here, the HDD was applied at concentrations ranging from 0.1 to 25%. It means that high concentrations are not needed to find effects when using HDD.

Even more important than the concentration is the dynamization tested, which can give different results even for the same HDD. Modolon *et al*. (2017) tested different dynamizations such as 12CH, 36CH, 60CH, and 84CH of *Silicea terra* aiming to reduce the *Spodoptera frugiperda* caterpillars' damage in maize plants. They found that the dynamization 36CH was the best to reduce food ingestion by the caterpillars. *Rupp et al*. (2012) sprayed HDD in the field during peach maturation, aiming to reduce the infestation by the true fruit fly *Anastrepha fraterculus*. They observed that, during the first harvest, *Staphysagria* 6CH applied every 10 days resulted in lower fruit fly infestation compared to *Staphysagria* 3CH under the same conditions. The next phase of this study involves selecting HDDs that have shown promising results, such as *Bufo rana*, *Lilium tigrinum*, and *D. suzukii* nosode, for further exploration using various dynamizations, beginning with 12CH.

6.6 CONCLUSION

Our results indicate that HDD can indeed influence the reproduction of *D. suzukii.* Adding HDD to a sugar solution generally stimulated the consumption of the sugar solution and also resulted in increased reproduction for two of the tested HDD, *Hyosciamus niger* 19CH and *Bufo rana* 19CH. When flies were given a substrate containing HDD, they produced more offspring on a substrate with *D. suzukii* nosode 19CH, *Hyosciamus niger* 19CH, and *Sepia succus* 19CH. The F1 progeny that developed on these substrates containing HDD then showed also increased egg-laying when they reached adulthood. This illustrates that the life stage (adults and larvae) exposed to the HDD can yield different results, even when using the same HDD. These findings highlight the complex and multifaceted nature of HDD's effects on *D. suzukii*, and further research may unveil additional nuances of this relationship.

Indeed, our findings presented unexpected results, as they revealed an increase in the reproduction of *D. suzukii*, both when measured as adult emergence and in egg-laying assays. While this outcome was not aligned with our original goal of developing HDD for pest management, it opens up intriguing possibilities for the use of HDD. Specifically, this increase in *D. suzukii* reproduction suggests that HDD could have applications in the field of insect mass rearing. This is especially relevant considering the recent growth of this sector within the animal feed industry.

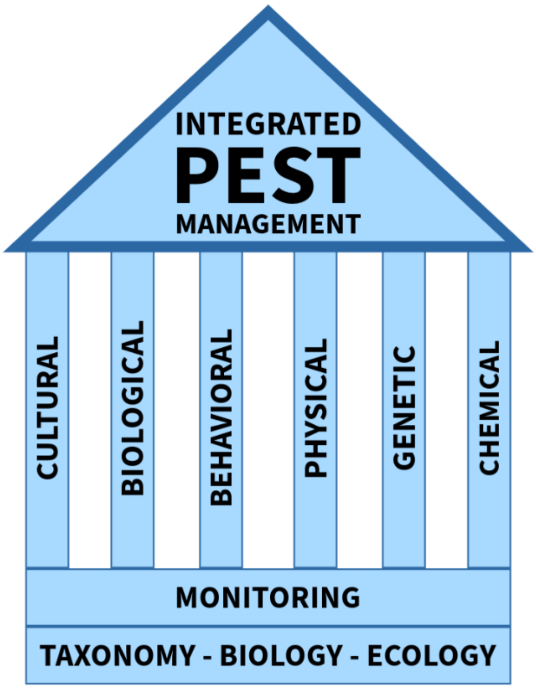
We do not know yet whether *D. suzukii* is attracted to HDD. The increased consumption of a sugar solution with HDD could suggest this. This could be explored to see if adding HDD in traps and lures would increase insect capture in the field for monitoring or mass trapping. In addition, HDD could be utilized as phago-stimulatory additive for baits that can be combined with toxic substances, to enhance their uptake. For crop protection, however, the tested HDD do not seem to be promising candidates, as they did not result in repelling *D. suzukii* from either feeding or oviposition.

7 GENERAL DISCUSSION

7.1 INTEGRATED MANAGEMENT OF *Drosophila suzukii*

The Food and Agriculture Organization on the United States (FAO) defines Integrated Pest Management (IPM) as: "*the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM promotes the growth of a healthy crop with the least possible disruption to agroecosystems and encourages natural pest control mechanisms*" (FAO 2023). This comprehensive framework employs a range of control techniques, including cultural, biological, behavioral, physical, genetic, and chemical methods, with decision-making based on monitoring (Figure 15).

Figure 15 - Structure of the integrated pest management.



Source: Author (2024)

IPM provides a solid theoretical foundation for pest control. However, its practical implementation faces challenges, with gaps particularly evident in ecological science development (Deguine *et al.*, 2021). The studies developed in this thesis aimed to address some of these gaps through a combination of field and laboratory work focused on developing knowledge for the integrated management of *Drosophila suzukii* (spotted-wing drosophila – SWD). Although these efforts contribute significantly, they alone are insufficient to formulate a comprehensive management program. Therefore, this general discussion outlines the essential steps for integrated *D. suzukii* management, especially in vineyards in southern Brazil. This integration involves synthesizing the findings presented here with additional ecological research focused on *D. suzukii* management.

The first step of *Drosophila suzukii* management involves the studies of monitoring to detect the presence of the species in the field. In the vineyards located in the highland region of Santa Catarina state in Brazil, using different types of traps and attractive baits, the presence of *D. suzukii* adults was first reported during the 2015/16 season (Padilha *et al.*, 2016). In the same season, reports from winemakers highlighted observations of drosophilid larvae during the winemaking process of Cabernet Sauvignon, Merlot, and Sangiovese grapes. As winemaking is of high economic importance to the region, this clearly indicated that it was necessary to do and develop research to better understand the population dynamic of *D. suzukii* in the vineyards in the highland region of Santa Catarina state in Brazil.

Understanding the population dynamics of *D. suzukii* involves comprehensive monitoring and identification of preferable habitats, favorable climate conditions, and potential fruit hosts. Through monitoring of *D. suzukii* over two years (**Chapter II**), and across three wine grape seasons (**Chapter III**), in four commercial Cabernet Sauvignon vineyards and in an experimental vineyard, we obtained results that showed that *D. suzukii* was detected and is present both inside the vineyard and in the surrounding forest border. We found that *D. suzukii* adults had higher population density, even up to ten times more, in the forest border than inside the vineyard. This observation aligns with findings reported in the literature that *D. suzukii* prefers the surrounding vegetation (Briem *et al.*, 2018; Groot *et al.*, 2021; Ioriatti *et al.*, 2015; Mazzetto *et al.*, 2020; Santoiemma *et al.*, 2019; Weißinger *et al.*, 2019a; 2019b). However, during the grape ripening, females tended to migrate into the vineyards. This migration can be attributed to the volatiles released by the grapes, attracting females seeking suitable locations for oviposition. Additionally, the ripening period is particularly susceptible to *D. suzukii* infestation, given the decreased skin resistance as harvest approaches (Ioriatti *et al.*, 2015).

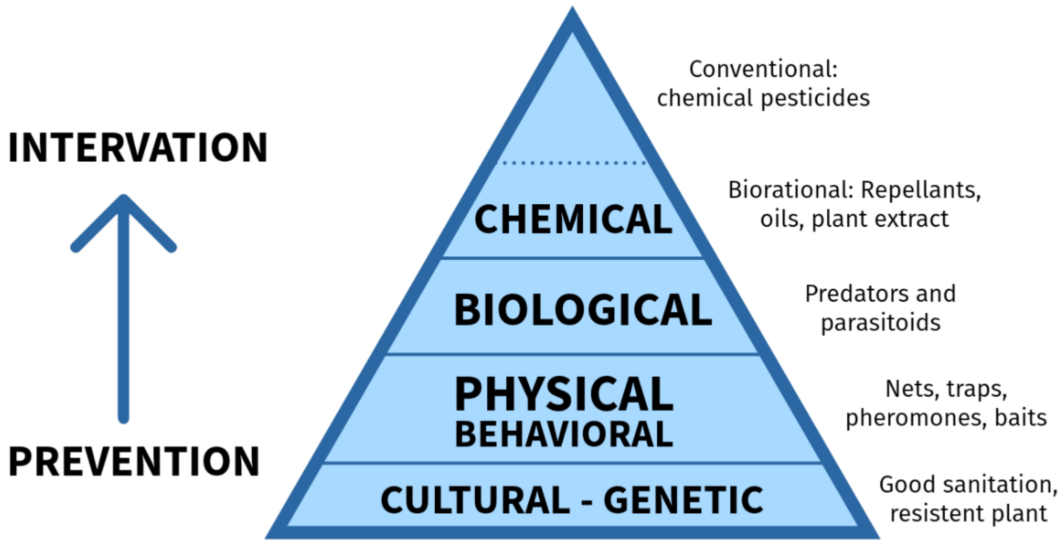
Using regression models, we identified which climate variable had the most significant explanatory value for the *D. suzukii* numbers in the vineyards of the highland region of Santa Catarina state. While the population of *D. suzukii* initiates its increase during spring with rising temperatures, our findings from **Chapters II and III** indicate that relative humidity explains a substantial portion of the variation in trap captures of *D. suzukii*, with high values of approximately 80% correlating with high catch rates. Moreover, variations in temperature (maximum, average, and minimum), rainfall, and thermal range differed for each vineyard, emphasizing the fact that each vineyard has its own microclimate (Wiman *et al.*, 2014). Additionally, it is important to highlight that the south region of Brazil experiences the quasi-periodic phenomena of "El Niño" and "La Niña," leading to periods of rain and high humidity or dry spells, respectively (Shi *et al.*, 2023). The last El Niño occurred between 2014 and 2016, which is the same period when the first *D. suzukii* was reported in the Cabernet Sauvignon, as well as the presence of larvae during the winemaking process (Arioli; Botton; Bernardi, 2015; Padilha *et al.*, 2016). Our monitoring experiments of *D. suzukii* started in the season of 2018/19, marked by a high *D. suzukii* capture rate (**Chapters II and III**). However, in the following season (2019/2020) we observed a drastic reduction in the capture rates (**Chapters II and III**) which might be related to the last occurrence of the La Niña (dry season) that started in 2020 and ended in 2023. It is plausible that these quasi-periodic phenomena may contribute to *D. suzukii* population fluctuations since they primarily affect relative humidity. Nevertheless, this should be better investigated in future monitoring research.

Although we did not specifically identify the host preferences of *D. suzukii* in this thesis, the surrounding forest borders of the vineyards are known to be composed of mostly native fruit plant species, with Myrtaceae species being predominant (Higuchi *et al.*, 2013). Myrtaceae species, constituting 23% of reported hosts to *D. suzukii* in South America, are likely attractive to *D. suzukii* (Kirschbaum et al., 2020). Additionally, in the same region and nearby the vineyards, small to medium orchards of host plants such as blackberries, blueberries, strawberries, and raspberries are cultivated. These orchards are like green bridges, contributing to the supply of fruits and maintenance of the *D. suzukii* population (Andreazza *et al.*, 2017).

Given the knowledge that we gained on the population dynamics in and around vineyards, the farmers need to pay attention, especially during the "*veraison*" stage of grape ripening. This is when the *D. suzukii* adults were present in the highest numbers both outside and inside the vineyards. Moreover, in non-choice laboratory assays with different grape cultivars, we showed that all grape varieties are in principle susceptible to puncturing of the skin and oviposition (**Chapters III and IV**). This shows the potential of *D. suzukii* to cause damage and economic losses in grape production.

To develop a procedure for the monitoring and management of *D. suzukii* in vineyards, we first need to recognize that a vineyard is a complex ecosystem with interrelationships between plants, animals, cultural operations, and microclimate. An Integrated Pest Management program should therefore combine control methods within a practical operational framework (Mani; Shivaraju; Kulkarni, 2014). There is a hierarchy in interventions when monitoring does reveal the presence of the pest insects, as presented in Figure 16. Cultural techniques should be the first control method to be applied, followed by physical and behavioral measures, then biological measures, and as a last resort chemical control, if it is necessary.

Figure 16 - Integrated pest management pyramid with the hierarchy of prioritization of the control techniques.



Source: Author (2024)

Cultural techniques, applicable throughout the year, play a crucial role in Integrated Pest Management. Sanitation of the vineyards is an essential practice for reducing population increases from one season to the next. This practice includes clearing the ground to remove microhabitats, pruning to open up the canopy, thereby enhancing airflow, and reducing shading and humidity in the grape bunches during the ripening period (Haye *et al.*, 2016; Schöneberg *et al.*, 2020). Soil and nutrition management contribute to maintaining vine balance, and ensuring healthy grape bunches by avoiding overly vigorous or weak plants (Mani; Shivaraju; Kulkarni, 2014; Provost; Pedneault, 2016). Sanitation includes also the management of the surrounding vegetation (Tait *et al.*, 2021). **Chapters II and III** of this thesis revealed that the forest border is a preferred habitat for *D. suzukii*. However, we do not recommend the removal of these native forest fragments, both because it is forbidden by Brazilian law (Law nº 12.651, 25 May 2012) (Brasil, 2013) and due to their role as a shelter for numerous beneficial insects and natural enemies. Additionally, native forests contribute to the conservation of plant and animal species (Tscharntke *et al.*, 2007). Instead of removal, managing this area to maintain *D. suzukii* and reduce migration into the vineyards during grape ripening could be explored. Further ecological studies are necessary for this purpose.

The management of the harvest period is also important, where farmers should condense to reduce/avoid *D. suzukii* infestation (Haye *et al.*, 2016). However, condensing the harvest period is difficult since wine grape cultivars mature at different times within the same season (Shrader; Burrack; Pfeiffer, 2019). Thus, monitoring grape aspects (physical/chemical) to determine the best time to harvest may help to avoid *D. suzukii* infestations. In **Chapter IV**, an investigation into the physical-chemical characteristics of grapes revealed that penetration resistance is a physical characteristic key to influencing *D. suzukii* infestation in grapes, corroborating the statement that it is a reliable predictor for *D. suzukii* attack (Baser *et al.*, 2018; Entling *et al.*, 2019; Pelton; Gratton; Guédot, 2017). The direct measurement of penetration resistance might be impractical for farmers themselves since it requires high-priced equipment to analyze the skin resistance. However, studies indicate that as grapes approach the harvest period, penetration resistance tends to decrease making the grapes more susceptible to *D. suzukii* attacks (Ioriatti *et al.*, 2015). Thus, vigilance needs to increase when grapes approach harvest time. This result could be aligned with other grape wine parameters measured by the farmers to signal and manage the harvest period. Usually, Brazilian farmers determine the harvest period based on sugar content (ºBrix), which our study showed to be correlated with the increase in the number of eggs laid by *D. suzukii* females (**Chapter IV**). Therefore, farmers can use this chemical parameter to guide the harvest period as it aligns with grape ripeness, while also signaling when the grapes are most susceptible to *D. suzukii* infestation. Additionally, during the harvest period, the removal of dropped and overripe grape berries is essential to reduce late-season reproductive resources and the next *D. suzukii* generations (Bal; Adams; Grieshop, 2017). This is important, because *D. suzukii* can infest both healthy and damaged fruits (**Chapter III and IV**).

Late-cycle cultivars can be at greater risk of *D. suzukii* oviposition since the fly populations at the end of the summer are larger than in early summer (Shrader; Burrack; Pfeiffer, 2019). Our findings in **Chapter IV** highlight that wine grape cultivars Chardonnay and Cabernet Sauvignon are most susceptible to *D. suzukii* oviposition in laboratory assay infestation. However, considering the typical harvest periods in the highland region of Santa Catarina state, Chardonnay is one of the first cultivars to be harvested and therefore potentially escaping significant *D. suzukii* damage. On the other hand, Cabernet Sauvignon, being one of the latest harvested cultivars, may face a greater risk of *D. suzukii* infestation.

Physical control measures, such as the use of nets to prevent *D. suzukii* infestation in berries, have been explored and recommended in the literature (Del Fava; Ioriatti; Melegaro, 2017; Ebbenga *et al.*, 2019). Studies conducted in vineyards in Michigan, U.S.A., demonstrated that the use of nets led to a remarkable reduction of approximately 95% in the average number of larvae/adults per berry, proving to be more effective than conventional chemical control methods (Ebbenga *et al.*, 2019). Moreover, nets offer additional benefits by reducing injuries caused by various factors, including other animals (wasps, bees, and birds) and abiotic elements such as wind, hail, and heavy rains (Ebbenga *et al.*, 2019). Female *D. suzukii* then can lay eggs in injuries, as observed in **Chapters III and IV**. Taking into account that nets need high initial capital investment, it can be justified in organic production (Provost; Pedneault, 2016) or the production of high-value wine cultivars (Figure 17). This may not be the case for widely produced cultivars like Cabernet Sauvignon and Merlot in the highland area of Santa Catarina state, where these two cultivars constitute a significant portion of the production (29.4% and 15%, respectively) (Pandolfo; Vianna, 2020). The decision to employ nets ultimately rests with the farmers, who may consider factors such as economic viability and specific production goals. In this case, the nets should be mounted and closed in the field during the "*veraison*" period (Ebbenga *et al.*, 2019).

Figure 17 - Nets applied in vines conducted in an espalier system to protect grape bunches from animal and mechanical damage during the grape ripening period. Videira Experimental Station of the Santa Catarina Agricultural Research and Rural Extension Company, Videira, Santa Catarina, Brazil.

Source: Fiedler (2023).

Among the behavior-based control methods, mass trapping and toxic baits can be an option in managing *D. suzukii.* These are strategies of push-pull and attract-and-kill (Tait *et al.*, 2021). Mass trapping involves the use of a high density of traps with liquid or solid attractants to capture insects, thereby protecting crops from damage (Brilinger *et al.*, 2018). This approach can be part of the integrated management of *D. suzukii*, contributing to a reduction in insecticide applications (Clymans *et al.*, 2022). However, the efficacy of mass trapping depends strongly on the attractiveness of the lure used (Lee *et al.*, 2013). A study conducted in Cabernet Sauvignon vineyards in the highland area of Santa Catarina state, testing a combination of traps and food attractants, showed that the attractive Droskidrink (red wine, apple cider vinegar, and sugar), provided the highest capture rates of *D. suzukii* (Brilinger *et al.*, 2021). This was the same attractant that we used in **Chapters II and III**. This food attractant proved also to have the lowest non-target effects and selective against hymenopteran and coleopterans. In a cherry orchard in Belgium, researchers evaluated the viability of mass trapping for *D. suzukii* control, estimating trap densities ranging from 75 to 200 traps/ha during the spring and 90 to 300 traps/ha during the summer, baited with apple cider vinegar (Clymans *et al.*, 2022). This technique holds potential for application in vineyards during the "*veraison*" stage, when grapes become susceptible to *D. suzukii*. Implementing mass trapping in the forest border could also be explored to keep *D. suzukii* within this natural habitat and prevent migration into the vineyards. However, further research is needed to test its effectiveness of mass trapping and determine the optimal trap density for this specific environment and purpose.

In **Chapter VI** of this study, we showed that some high dynamized dilution (HDD) added to a 20% sugar solution increased the consumption of the solution by *D. suzukii* females. Future research could explore the incorporation of HDD into either the attractive Droskidrink could increase catching capacity and lengthen attractiveness time in capturing *D. suzukii*. For example, adding HDD of *Acidum tannicum* 30CH to grape juice bait resulted in increased captures of *Anastrepha fraterculus* (Diptera: Tephritidae) in a feijoa orchard (Brilinger *et al.*, 2018). Alternatively, it may be a useful additive in toxic baits, to stimulate the ingestion of the baits. Also, some types of homemade toxic baits, ready-to-use and lures were tested on *D. suzukii* adults under laboratory conditions (Brilinger *et al.*, 2024). The authors identified that adding 0.3% sugar solution into the lure Droskidrink + 0.15% malathion could reach up to 65% of mortality rate. However, further studies need to be developed to assess the persistence of this toxic bait in conditions of semi-field and fields.

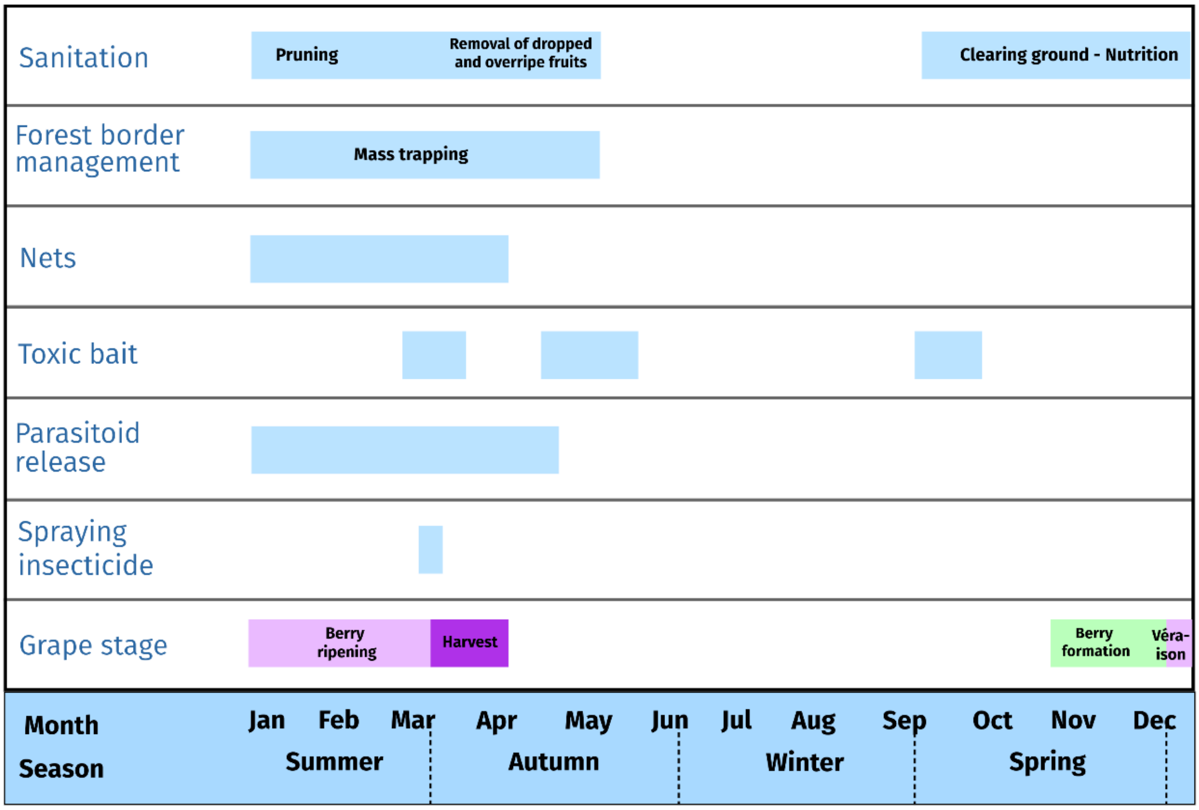
In **Chapter V**, we investigated a botanical compound extract from the medicinal Chinese herb *Sophora flavescens*, which has demonstrated insecticidal effects, particularly against lepidopterans and aphids (Xu *et al.*, 2020; Zanardi *et al.*, 2015). Our findings indicated that this botanical compound also has the potential to be integrated into the management of *D. suzukii*, showing both mortality effects and a reduction in insect movement (commercial matrine-based product). The intended use of this botanical compound in *D. suzukii* management is in a toxic bait strategy, combined with a specific food substance to attract *D. suzukii*. The use of toxic bait can reduce the amount of applied insecticide and minimize pesticide residues in fruits, as it can be applied onto the leaves rather than the fruits (Klick *et al.*, 2019). Additionally, since the toxic bait composition can be tailored to specifically target *D. suzukii*, it may help avoid affecting beneficial insects, contributing to the maintenance of ecosystem balance (Klick *et al.*, 2019). Recent studies have explored toxic bait formulations for *D. suzukii* containing mainly Spinosad as a lethal agent (Babu; Rodriguez-Saona; Sial, 2022; Klick *et al.*, 2019; Rice; Short; Leskey, 2017; Wise *et al.*, 2018). However, to mitigate the risk of insect resistance, the use of Spinosad must be limited in the number of applications per year (Haye *et al.*, 2016). In this context, the matrine-based insecticide could be used as an alternative in a toxic bait rotation with Spinosad. This approach could be implemented in the forest border, both at the beginning of spring when the SWD population starts to increase (**Chapters II and III**) and after the harvest period to reduce the emergence of the next generation after winter. Furthermore, it could be combined with mass trapping during the grape ripening period, with mass trapping applied to the forest border and the toxic bait applied in the borders of the vineyards to create a barrier.

Even though the experiments developed in this thesis did not involve research into biological control, it is important to emphasize the importance of incorporating the biological control measure into the *D. suzukii* integrated management program. As stated by Haye *et al*. (2016) and Tait *et al*. (2021) the use of predators, parasitoids, and/or entomopathogens is considered cost-effective and environmentally safe for managing arthropod pests. In Latin America, the focus of biological control has primarily been on parasitoid wasps. Among the 14 identified species, *Trichopria anastrephae* (Hymenoptera: Diapriidae) has shown promise as a *D. suzukii* parasitoid in Brazil (Garcia *et al.*, 2022). The *T. anastrephae* wasp can parasitize *D. suzukii* pupae, can be easily reared in the laboratory, and is less sensitive to insecticides, making it more feasible to add it to an integrated management program (Schlesener *et al.*, 2019; Vieira *et al.*, 2020). Furthermore, during the monitoring period, as presented and discussed in **Chapters II and III**, we also captured hymenopteran specimens. Although the collected specimens have not yet been sent for identification, they may reveal the occurrence of natural enemies of *D. suzukii* in vineyards cultivated in the highland area of Santa Catarina state, Brazil. Therefore, we recommend conducting a study to identify the parasitoid species that are associated with *D. suzukii* in vineyards. This information could contribute significantly to the development of effective biological control strategies.

Regarding chemical control, many farmers still consider it the first measure to prevent *D. suzukii* infestation (Haye *et al.*, 2016), even though it should ideally be the last resort according to the IPM pyramid. Broad-spectrum insecticides are often applied using calendar scheduling based on the duration of protection provided by the molecules (Van Timmeren; Isaacs, 2013). In Brazil, the insecticide Spinosad is currently the only authorized substance by the Brazilian Ministry of Agriculture, Livestock, and Supply for controlling *D. suzukii* in blackberry, blueberry, raspberry, and grape (MAPA, 2023). However, insecticide applications during the pre-harvest period are challenging for fruit production due to the maximum residue limits allowed for fruits (Diepenbrock; Swoboda-Bhattarai; Burrack, 2016; Haviland; Beers, 2012). Spraying insecticides at approved rates before harvest may not effectively reduce infestation damage to fruits, especially when larvae are inside the fruits and difficult to reach by the insecticides (Gargani *et al.*, 2013). A study conducted in wild blueberry orchards suggested that even a risk-based action, such as spraying insecticides in a cover area to control *D. suzukii*, may not be the most effective option for farmers across various management scenarios (Yeh *et al.*, 2020). This emphasizes the importance of adopting a holistic and multifaceted approach, integrating cultural (sanitation), physical (nets), behavioral (attract and kill, mass trapping, high-dynamized dilutions), and biological (parasitoids) control measures in the overall management strategy for *D. suzukii*.

Considering the results obtained and documented in this thesis, together with the observations and information obtained during field research and literature review activities, I propose a timeline for year-round the integrated management of *D. suzukii* in vineyards located in the highland area of Santa Catarina state (Figure 18). Sanitation measures should be implemented, regardless of the climatic conditions, all along the cultivation season. Ideally, this should also include collecting fallen and remaining fruits before, during, and after harvest, to limit the reservoir of breeding sites during the post-harvest period. To reduce the population that will go through diapause, toxic bait application could be done before winter and at the beginning of spring when the *D. suzukii* population becomes active again. Mass trapping could be applied in the forest border during the ripening period to keep the *D. suzukii* numbers low in this habitat. Additionally, the use of toxic baits can be applied at the vineyard border during the pre-harvest period to prevent the entry of *D. suzukii* females. Also, keeping the *D. suzukii* inside of the forest border can contribute to the maintenance of natural enemies populations, such as parasitoids, that occur naturally in this habitat. Additionally, parasitoid (*Trichopria anastrephae*) releases could be scheduled during the season in the forest border or in *D. suzukii* host plants near the vineyard border, starting after the "*veraison*" period and concluding after the harvest. Insecticide spraying should be considered as a last control option during the pre-harvest period if infestation in the grape berries is identified. If the farmer opts to use nets, it provides an alternative to mass trapping, parasitoid release, toxic bait, and insecticide spray during the ripening period. However, sanitation measures and forest border management should still be conducted to reduce the *D. suzukii* population and minimize the risk of *D. suzukii* attacks.

Figure 18 - Timeline proposed for year-round integrated management of *Drosophila suzukii* in vineyards located in the highland area of Santa Catarina State, Brazil.



Source: Author (2024)

7.2 GENERAL CONCLUSIONS

The spotted-wing drosophila (SWD) has established itself in Brazil. It could develop as a pest in vineyards situated in the highland region of the Santa Catarina state, Brazil. The adults of *D. suzukii* show a preference for the borders consisting of native forests, but during the ripening period, females tend to migrate into the vineyard. This constitutes a significant risk of *D. suzukii* infestations in vineyards, but only during relatively short periods of the year, and specifically during the grape ripening period. The climate variable most closely associated with the increase in *D. suzukii* population in the vineyards is relative humidity, particularly when it is high, around 80%. This climatic variable can be used as a reliable predictor for *D. suzukii* population growth.

Wine grape cultivars present different levels of susceptibility to *D. suzukii* attack. Sangiovese proved to be more susceptible in a free-choice test during the 2020/2021 season, while Cabernet Sauvignon was highly susceptible in a non-choice test involving undamaged and damaged berries during the 2021/2022 season. The physical characteristic of skin resistance, specifically high penetration resistance, was identified as conferring resistance to *D. suzukii* attacks in grape wine cultivars. However, in cases where the grape is damaged, the chemical characteristic of pH provides resistance to oviposition and adult emergence. These findings can be used by the farmers, enabling them to implement *D. suzukii* management strategies selectively in vineyards where the cultivar is susceptible.

The development of sustainable tools to manage *D. suzukii* in the field is crucial. Matrine-based insecticide can be a sustainable alternative to insecticide since it is derived from a plant extract and serves as a potential substitute for spinosad. This is especially relevant for organic farmers, as spinosad is currently the primary insecticide used to manage *D. suzukii* in the field. The addition of high dynamized dilution affected the behavior of *D. suzukii* females, with increased consumption and egg laying. This can be further explored to see if it is also attractive, and could increase insect capture in traps and lures, contributing to effective monitoring and mass trapping strategies. Furthermore, the use of some HDD showed promise as a phagostimulant, which could enhance the uptake of toxic baits. As repellent, however, the tested HDDs do not show good potential for the control of *D. suzukii*. Incorporating these sustainable tools into

SWD management strategies can contribute to environmentally friendly and targeted pest control practices.

The results presented in this thesis underscore the importance of developing strategies aimed at suppressing the *D. suzukii* population in vineyards. Furthermore, it is important to develop tools that are accepted in an integrated and organic fruit production system. This approach is essential to ensuring food security and maintaining the health of agroecosystems.

BIBLIOGRAPHY

ABBOTT, W. S. A method of computing the effectiveness of on insecticide. **Journal Economic Entomology**, v. 18, n. 2, p. 265-267, 1925.

ALI, S. *et al.* Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). **Scientific Reports**, v. 7, p. 1–14, 2017.

ALMADANI, A. H.; HIWARE, C. J. The effect of homeopathic drug and essential oil against greater wax moth, *Galleria mellonella* L. **Indian Journal of Agricultural Research**, v. 54, n. 4, p. 477–482, 2020.

ANDREAZZA, F. *et al. Drosophila suzukii* in Southern Neotropical Region: Current Status and Future Perspectives. **Neotropical Entomology**, v. 46, n. 6, p. 591–605, 2017.

ANDREAZZA, F. *et al.* Suscetibilidade de bagas de genótipos de videira pela infestação por *Drosophila suzukii* (Diptera: Drosophilidae). **Pesquisa Agropecuária Brasileira**, v. 51, n. 5, p. 599–606, 2016.

ANDREAZZA, F. *et al.* Técnica de criação de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). **Boletim de Pesquisa,** Embrapa Clima Temperado, n. 9, p. 23, 2016.

ARA, Z. G.; HAQUE, A. R. A Comprehensive Review on Synthetic Insecticides: Toxicity to Pollinators, Associated Risk to Food Security, and Management Approaches. **Journal of Biosystems Engineering**, v. 46, n. 3, p. 254–272, 2021.

ARIOLI, C. J.; BOTTON, M.; BERNARDI, D. *Drosophila suzukii*: uma possível praga nos vinhedos da Serra Catarinense. **A Tribuna**, São Joaquim, Oct. 2015. p. 17–21.

ASPLEN, M. K. *et al.* Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. **Journal of Pest Science**, v. 88, n. 3, p. 469–494, 2015.

ATALLAH, J. *et al.* The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. **Proceedings of the Royal Society B: Biological Sciences**, v. 281, n. 1781, 2014.

AVHAD, S. B.; PAGARE, P. M.; HIWARE, C. J. Impact Assessment of Homeopathic Drugs Iodium and Lachesis on Larvae Cocoon and Post-Cocoon Characteristic of *Bombyx mori* L. **International Journal of Research Studies in Biosciences**, v. 3, n. 4, p. 93–97, 2015.

BABU, A.; RODRIGUEZ-SAONA, C.; SIAL, A. A. Factors Influencing the Efficacy of Novel Attract-and-Kill (ACTTRA SWD) Formulations Against *Drosophila suzukii*. **Journal of Economic Entomology**, v. 115, n. 4, p. 1–9, 2022.

BAENA, R. *et al.* Ripening stages and volatile compounds present in strawberry fruits are involved in the oviposition choice of *Drosophila suzukii* (Diptera: Drosophilidae). **Crop Protection**, v. 153, p. 105883, 2022.

BAL, H. K.; ADAMS, C.; GRIESHOP, M. Evaluation of Off-season Potential Breeding Sources for Spotted Wing Drosophila (*Drosophila suzukii* Matsumura) in Michigan. **Journal of Economic Entomology**, v. 110, n. 6, p. 2466–2470, 2017.

BARATA, A.; MALFEITO-FERREIRA, M.; LOUREIRO, V. Changes in sour rotten grape berry microbiota during ripening and wine fermentation. **International Journal of Food Microbiology**, v. 154, n. 3, p. 152–161, 2012.

BARZMAN, M. *et al.* Eight principles of integrated pest management. **Agronomy for Sustainable Development**, v. 35, n. 4, p. 1199–1215, 2015.

BASER, N. *et al.* Susceptibility of table grape varieties grown in south-eastern Italy to *Drosophila suzukii*. **Journal of Applied Entomology**, v. 142, n. 5, p. 465–472, 2018.

BATES, D. *et al.* Fitting linear mixed-effects models using lme4. **Journal of Statistical Software**, v. 67, n. 1, 2015.

BEGON, M.; TOWNSEND, C. R. **Ecology: from individuals to ecosystems**. Nova Jersey: John Wiley & Sons, 2021.

BELLAMY, D. E.; SISTERSON, M. S.; WALSE, S. S. Quantifying Host Potentials: Indexing Postharvest Fresh Fruits for Spotted Wing Drosophila, *Drosophila suzukii*. **PLoS ONE**, v. 8, n. 4, 2013.

BENITO, N. P.; LOPES-DA-SILVA, M.; SANTOS, R. S. S. dos. Potential spread and economic impact of invasive *Drosophila suzukii* in Brazil. **Pesquisa Agropecuaria Brasileira**, v. 51, n. 5, p. 571–578, 2016.

BERRY, J. A. **Pest Risk Assessment: *Drosophila suzukii*: spotted wing drosophila (Diptera: Drosophilidae) on fresh**. Wellington: Ministry for Primary Industries, New Zealand Government, 2012.

BERRY, J. A. *Drosophila suzukii:* pathways and pathway management by regulation. In: GARCIA, F.R.M. ***Drosophila suzukii* Management**. Springer, 2020.

BLOOMQUIST, J. R. *et al.* Insecticidal activity and physiological actions of matrine, a plant natural product. **ACS Symposium Series**, v. 1289, p. 175–186, 2018.

BLOUQUY, L. *et al.* How varying parameters impact insecticide resistance bioassay: An example on the worldwide invasive pest *Drosophila suzukii*. **PLoS ONE**, v. 16, n. 3, p. 1–19, 2021.

BOFF, P.; VERDI, R.; FAEDO, L. F. Homeopathy Applied to Agriculture: Theoretical and Practical Considerations with Examples from Brazil. In: WRIGHT, J. **Subtle Agroecologies**. CRC Press, 2021. p. 145-154.

BOLDA, M. P.; GOODHUE, R. E.; ZALOM, F. G. Spotted Wing Drosophila: potential economic impact of a newly established pest. **Agricultural and resource economics**, v. 13, n. 3, p. 5–8, 2010.

BOUGHDAD, A. *et al.* First record of the invasive spotted wing Drosophila infesting berry crops in Africa. **Journal of Pest Science**, v. 94, n. 2, p. 261–271, 2021.

BRASIL. **Farmacopeia Homeopática Brasileira**. Brasília: ANVISA, v. 3, 2011. Available at: http://portal.anvisa.gov.br/documents/33832/259147/3a_edicao.pdf/cb9d5888-6b7c-447b-be3c-af51aaae7ea8.

BRASIL. **Lei nº 20.922, de 16 de outubro de 2013**. Código Florestal. Brasília, 2013. Available at: http://www.planalto.gov.br/ccivil03/leis/L4771.htm.

BRIEM, F. *et al.* Explorative data analysis of *Drosophila suzukii* trap catches from a seven-year monitoring program in Southwest Germany. **Insects**, v. 9, n. 4, 2018.

BRILINGER, D. *et al.* Captura massal: uma ferramenta no manejo de moscas- das-frutas. **Revista da 15a Jornada de Pós graduação e Pesquisa**, v. 15, n. 15, p. 821–834, 2018.

BRILINGER, D. *et al. Drosophila suzukii* population dynamics in vineyards and wine cultivars susceptibility. **Revista Brasileira de Fruticultura**, v. 45, p. e-989, 2023.

BRILINGER, D. *et al.* Effect of toxic baits on adult spotted - wing drosophila under laboratory conditions. **Revista Caatinga**, v. 37, p. e11977, 2024.

BRILINGER, D. *et al.* Efficiency of attractors and traps for capture of spotted-wing drosophila in vineyards. **Revista Caatinga**, v. 34, n. 4, p. 830–836, 2021.

BRILINGER, D. *et al.* Preparados homeopáticos no monitoramento de moscas-das-frutas em pomar de goiabeira-serrana (*Acca sellowiana*). *In*: Anais do Congresso de Homeopatia nas Ciências Agrárias e do Ambiente. **Anais** [...] Ribeirão Preto: Instituto Homeopático e de Práticas Integradas, 2018. p. 11–12.

BURRACK, H. J. *et al.* 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**, v. 69, n. 10, p. 1173–1180, 2013.

CAHENZLI, F.; STRACK, T.; DANIEL, C. Screening of 25 different natural crop protection products against *Drosophila suzukii*. **Journal of Applied Entomology**, v. 142, n. 6, p. 563–577, 2018.

CALABRIA, G. *et al.* First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. **Journal of Applied Entomology**, v. 136, n. 1–2, p. 139–147, 2012.

CAMARGO, U. A.; TONIETTO, J.; HOFFMANN, A. Advances in grape culture in Brazil. **Revista Brasileira de Fruticultura**, v. 33, n.1, p. 144–149, 2011.

CARVALHO, C. de; KIST, B. B.; BELING, R. R. **Anuário Brasileiro de Horti & Fruit**. Santa Cruz do sul: Gazeta, 2020, p. 96.

CARSON, H. L. **The ecology of *Drosophila* breeding sites**. The Harold L. Lyon Arboretum Lecture, University of Hawaii, n. 2, p. 1-27, 1971.

CAZELLA, A. A.; SOUZA, M. C. de; NUNES, K. **Análise Socioeconômica e Ambiental de São Joaquim - SC: um estudo a partir da disciplina Vivência em Agricultura Familiar**. Florianópolis, 2019.

CELIZ, R. J. R.; UBAUB, L. T. Insecticidal effects of Matrine against flower thrips , *Thrips hawaiiensis* Morgan on ‘ Cavendish ’ banana. **Journal of South Pacific Agriculture**, v. 21, p. 9–17, 2019.

CHAGNON, M. *et al.* Risks of large-scale use of systemic insecticides to ecosystem functioning and services. **Environmental Science and Pollution Research**, v. 22, n. 1, p. 119–134, 2015.

CINI, A. *et al.* Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe. **Journal of Pest Science**, v. 87, n. 4, p. 559–566, 2014.

CINI, A.; IORIATTI, C.; ANFORA, G. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. **Bulletin of Insectology**, v. 65, n. 1, p. 149–160, 2012.

CLYMANS, R. *et al.* Mass Trapping *Drosophila suzukii*, What Would It Take? A Two-Year Field Study on Trap Interference. **Insects**, v. 13, n. 3, p. 1–23, 2022.

DALTON, D. T. *et al.* Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. **Pest Management Science**, v. 67, n. 11, p. 1368–1374, 2011.

DAM, D.; MOLITOR, D.; BEYER, M. Natural compounds for controlling *Drosophila suzukii*. A review. **Agronomy for Sustainable Development**, v. 39, n. 6, 2019.

DE ROS, G. *et al.* The economic impact of invasive pest *Drosophila suzukii* on berry production in the Province of Trento, Italy. **Journal of Berry Research**, v. 5, n. 2, p. 89–96, 2015.

DE ROS, G. *et al.* The potrential economi impact of *Drosophila suzukii* on small fruits production in Trentino Italy. **IOBC wprs**, v. 10, p. 1–4, 2013.

DEBONI, T. C. *et al.* Bioatividade de preparados homeopáticos e extratos vegetais sobre *Acanthoscelides obtectus* (Coleoptera: Bruchidae) em grãos de feijão armazenados. **Revista Brasileira de Agroecologia**, v. 212, n. 2, p. 52–58, 2017.

DEGUINE, J. P. *et al.* Integrated pest management: good intentions, hard realities. A review. **Agronomy for Sustainable Development**, v. 41, n. 3, 2021.

DEL FAVA, E.; IORIATTI, C.; MELEGARO, A. Cost–benefit analysis of controlling the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) spread and infestation of soft fruits in Trentino, Northern Italy. **Pest Management Science**, v. 73, n. 11, p. 2318–2327, 2017.

DELBAC, L. *et al.* Geographical area extension of *Drosophila suzukii* (Diptera: Drosophilidae) in Bordeaux vineyards. **IOBC-WPRS Bulletin**, v. 128, p. 28–36, 2017.

DEPRÁ, M. *et al.* The first records of the invasive pest *Drosophila suzukii* in the South American continent. **Journal of Pest Science**, v. 87, n. 3, p. 379–383, 2014.

DIEPENBROCK, L. M.; BURRACK, H. J. Variation of within-crop microhabitat use by *Drosophila suzukii* (Diptera: Drosophilidae) in blackberry. **Journal of Applied Entomology**, v. 141, n. 1–2, p. 1–7, 2017.

DIEPENBROCK, L. M.; HARDIN, J. A.; BURRACK, H. J. Season-long programs for control of *Drosophila suzukii* in southeastern U.S. blackberries. **Crop Protection**, v. 98, p. 149–156, 2017.

DIEPENBROCK, L. M.; SWOBODA-BHATTARAI, K. A.; BURRACK, H. J. Ovipositional preference, fidelity, and fitness of *Drosophila suzukii* in a co-occurring crop and non-crop host system. **Journal of Pest Science**, v. 89, n. 3, p. 761–769, 2016.

DIGIACOMO, G. *et al.* Economic Impact of Spotted Wing Drosophila (Diptera: Drosophilidae) Yield Loss on Minnesota Raspberry Farms: A Grower Survey. **Journal of Integrated Pest Management**, v. 10, n. 1, p. 0–5, 2019.

DRUMMOND, F.; BALLMAN, E.; COLLINS, J. Population Dynamics of Spotted Wing Drosophila (*Drosophila suzukii* (Matsumura)) in Maine Wild Blueberry (*Vaccinium angustifolium* Aiton). **Insects**, v. 10, 2019.

EBBENGA, D. N. *et al.* Evaluation of Exclusion Netting for Spotted-Wing Drosophila (Diptera: Drosophilidae) Management in Minnesota Wine Grapes. **Journal of Economic Entomology**, v. 112, n. 5, p. 2287–2294, 2019.

EBEN, A. *et al.* Response of *Drosophila suzukii* (Diptera: Drosophilidae) to extreme heat and dryness. **Agricultural and Forest Entomology**, v. 20, n. 1, p. 113–121, 2018.

ENTLING, W. *et al.* Berry skin resistance explains oviposition preferences of *Drosophila suzukii* at the level of grape cultivars and single berries. **Journal of Pest Science**, v. 92, n. 2, p. 477–484, 2019.

EPAGRI. **Banco de dados de variáveis ambientais de Santa Catarina**. Florianopolis: Epagri, 2020.

EVANS, R. K.; TOEWS, M. D.; SIAL, A. A. Impact of short- and long-term heat stress on reproductive potential of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). **Journal of Thermal Biology**, v. 78, p. 92–99, 2018.

FAO. **NSP – Integrated Pest Management, FAO definition**. 2023. Avaliable at: http:// www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/ipm/ en/.

FAQEERZADA, M. A. *et al.* Postharvest technologies for fruits and vegetables in South Asian countries: a review. **Agrcultural Science Korean Journal of Agricultural Science**, v. 45, n. 3, p. 325–353, 2018.

FARNSWORTH, D. *et al.* Economic analysis of revenue losses and control costs associated with the spotted wing drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry. **Pest Management Science**, v. 73, n. 6, p. 1083–1090, 2017.

FOPPA, F. *et al.* Ocorrência de *Drosophila suzukii* (Matsumura) (Diptera, Drosophilidae) na cultura do pessegueiro, em Farroupilha, na Serra Gaúcha, RS. **EntomoBrasilis**, v. 11, n. 3, p. 178–184, 2018.

FRAIMOUT, A. *et al.* Deciphering the routes of invasion of Drosophila suzukii by Means of ABC Random Forest. **Molecular Biology and Evolution**, v. 34, n. 4, p. 980–996, 2017.

FUNES, C. *et al.* La mosca de las alas manchadas, *Drosophila suzukii* (Matsamura). **Instituto Nacional de Tecnologia Agropecuaria- INTA**, 2018, p. 32.

GADALLAH, A. *et al.* The effect of teflubenzuron and oxymatrine on some biological aspects of *Sesamia critica* Led. (lepidoptera: noctuidae). **Journal of Plant Protection and Pathology**, v. 5, n. 7, p. 837–847, 2014.

Garcia, F. R. M. Introduction to *Drosophila suzukii* management. In: Garcia, F.R.M. (eds) ***Drosophila suzukii* Management**. Springer, 2020.

GARCIA, F. R. M. *et al. Drosophila suzukii* Management in Latin America: Current Status and Perspectives. **Journal of Economic Entomology**, v. 115, n. 4, p. 1008–1023, 2022.

GARGANI, E. *et al.* Notes on *Drosophila suzukii* Matsumura (Diptera Drosophilidae): Field survey in Tuscany and laboratory evaluation of organic products. **Redia**, v. 96, p. 85–90, 2013.

GEISLER, F. C. S. *et al.* Primeiro registro de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) para o estado do Paraná, Brasil e de novos hospedeiros. **Revista de Ciências Ambientais**, v. 9, n. 2, 2015.

GIESEL, A.; BOFF, M. I. C.; BOFF, P. Atividade de formigas cortadeiras Acromyrmex spp. submetidas a preparações homeopáticas. **Acta Scientiarum - Agronomy**, v. 34, n. 4, p. 445–451, 2012.

GIUNTI, G. *et al.* Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. **Biological Control**, v. 176, 105071, 2022.

GOODHUE, R. E. *et al.* Spotted wing drosophila infestation of California strawberries and raspberries: Economic analysis of potential revenue losses and control costs. **Pest Management Science**, v. 67, n. 11, p. 1396–1402, 2011.

GRASSI, A. *et al.* Development and efficacy of Droskidrink, a food bait for trapping *Drosophila suzukii*. *In*: **VIII Workshop on Integrated Soft Fruit Production**, IOBC-WPRS, 2014.

GRASSI, A. *et al.* Seasonal Reproductive Biology of *Drosophila suzukii* (Diptera: Drosophilidae) in Temperate Climates. **Environmental Entomology**, v. 47, n. 1, p. 166–174, 2018.

GROOT, M. *et al.* Agricultural landscape affects sex-specific differences in the abundance of *Drosophila suzukii* in raspberry orchards. **Journal of Applied Entomology**, v. 146, n. 1-2, p. 19-31, 2021.

GUÉDOT, C.; AVANESYAN, A.; HIETALA-HENSCHELL, K. Effect of temperature and humidity on the seasonal phenology of *Drosophila suzukii* (Diptera: Drosophilidae) in wisconsin. **Environmental Entomology**, v. 47, n. 6, p. 1365–1375, 2018.

HAMBY, K. A. *et al.* Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. **Journal of Pest Science**, v. 89, n. 3, p. 605–619, 2016.

HARRIS, D. W. *et al.* Seasonal monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) in a mixed fruit production system. **Journal of Asia-Pacific Entomology**, v. 17, n. 4, p. 857–864, 2014.

HAUSER, M., GAIMARI, S., DAMUS, M. Drosophila suzukii new to North America. **Fly Times**, v. 43, p. 12-15, 2009

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

HAVILAND, D. R.; BEERS, E. H. Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. **Journal of Integrated Pest Management**, v. 3, n. 2, p. 1–6, 2012.

HAYE, T. *et al.* Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. **Journal of Pest Science**, v. 89, n. 3, p. 643–651, 2016.

HELSEN, H.; VAN BRUCHEM, J.; POTTING, R. De suzuki-fruitvlieg *Drosophila suzukii*, een nieuwe plaag op fruit in Nederland. **Gewasbescherming**, v. 44, n. 3, p. 72–76, 2013.

HIGUCHI, P. *et al.* Potencial de fragmentos florestais inseridos dentro de floresta de exóticas para a restauração da floresta com Araucárias. **Ciencia Florestal**, v. 23, n. 1, p. 153–164, 2013.

IORIATTI, C. *et al. Drosophila suzukii* (Diptera: Drosophilidae) and Its Potential Impact to Wine Grapes During Harvest in Two Cool Climate Wine Grape Production Regions. **Journal of Economic Entomology**, v. 108, n. 3, p. 1148–1155, 2015.

IORIATTI, C. *et al. Drosophila suzukii* (Diptera: Drosophilidae) contributes to the development of sour rot in grape. **Journal of Economic Entomology**, v. 111, n. 1, p. 283–292, 2018.

JA, W. W. *et al.* Prandiology of *Drosophila* and the CAFE assay. **Proceedings of the National Academy of Sciences of the United States of America**, v. 104, n. 20, p. 8253–8256, 2007.

JAKOBS, R.; GARIEPY, T. D.; SINCLAIR, B. J. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. **Journal of Insect Physiology**, v. 79, p. 1–9, 2015.

JANG, M. *et al.* Biological activity of Myrtaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). **Pest management science**, v. 73, n. 2, p. 404–409, 2017.

JANUÁRIO, T. L. da S. *et al.* Danos à saúde humana e ao meio ambiente ocasionados pelo uso de agrotóxicos na produção de produtos agrícolas no Brasil. **Revista Ibero-Americana de Ciências Ambientais**, v. 12, n. 5, p. 462–477, 2021.

KANZAWA, T. Studies on Drosophila suzukii Mats. **Japanese Journal of Plant Protection**, v. 23, 1936.

KANZAWA, T. Studies on *Drosophila suzukii* Mats. **Review of Applied Entomology**, 29, 622, 1939.

KASAP, İ.; ÖZDAMAR, E. Population development of *Drosophila suzukii* (matsumura) (Diptera: Drosophilidae) in vineyards of çanakkale province. **Turkiye Entomoloji Dergisi**, v. 43, n. 1, p. 57–62, 2019.

KEHRLI, P. *et al.* Optimization of the sampling method to monitor *Drosophila suzukii* infestation in vineyards. **Journal of Applied Entomology**, v. 146, n. 4, p. 408–414, 2022.

KENIS, M. *et al.* Non-crop plants used as hosts by *Drosophila suzukii* in Europe. **Journal of Pest Science**, v. 89, n. 3, p. 735–748, 2016.

KIRSCHBAUM, D. S. *et al*. The biology and ecology of *Drosophila suzukii* (Diptera: Drosophilidae). In: Garcia, F.R.M. ***Drosophila suzukii* Management**. Springer, 2020.

KIST, B. B. *et al.* **Anuário Brasileiro da Fruticultura Brasileira 2018**. Santa Cruz do Sul: Gazeta, 2018.

KLICK, J. *et al.* Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae) management. **Journal of Insect Science**, v. 19, n. 1, p. 1–6, 2019.

KÖHLER, J. Globalization and Sustainable Development: Case Study on International Transport and Sustainable Development. **Journal of Environment and Development**, v. 23, n. 1, p. 66–100, 2014.

KOLISKO, E.; KOLISKO, L. **Agriculture of Tomorrow**, 2nd ed. Bournemouth: Kolisko Archive Publications, 1978.

KORDESTANI, M. *et al.* Proteus, Matrine, and Pyridalyl Toxicity and Their Sublethal Effects on *Orius laevigatus* (Hemiptera: Anthocoridae). **Journal of Economic Entomology**, v. 115, n. 2, p. 573–581, 2022.

KRÜGER, A. P. *et al.* Insecticide-mediated effects on mating success and reproductive output of *Drosophila suzukii*. **Ecotoxicology**, v. 30, n. 5, p. 828–835, 2021.

LEAHY, J. *et al.* Biopesticide oversight and registration at the U.S. Environmental Protection Agency. **ACS Symposium Series**, v. 1172, p. 3–18, 2014.

LEE, Jana C. *et al.* In Focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. **Pest Management Science**, v. 67, n. 11, p. 1349–1351, 2011.

LEE, Jana C. *et al.* The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. **Pest Management Science**, v. 67, n. 11, p. 1358–1367, 2011.

LEE, J. C. *et al.* Trap designs for monitoring D*rosophila suzukii* (Diptera: Drosophilidae). **Environmental Entomology**, v. 42, n. 6, p. 1348–1355, 2013.

LINDER, C. *et al.* Susceptibility of various grape cultivars to *Drosophila suzukii* and other vinegar flies. **IOBC-WPRS Bulletin**, v. 105, p. 219–224, 2014.

LIU, L. *et al.* Actions of quinolizidine alkaloids on *Periplaneta americana* nicotinic acetylcholine receptors. **Pest management science**, v. 64, p. 1222–1228, 2008.

LIU, S. *et al.* Oviposition suitability of *Drosophila suzukii* (Diptera: Drosophilidae) for nectarine varieties and its correlation with the physiological indexes. **Insects**, v. 10, n. 8, p. 221, 2019.

MANI, M.; SHIVARAJU, C.; KULKARNI, N. S. **The grape entomology**. Springer New Delhi, 2014.

MANKO, P.; DE FIGUEROA, J. M. T.; OBOŇA, J. Responses of the pest *Drosophila suzukii* population to environmental variables in southern Spain (Granada, Andalusia) – results of a pilot study. **Acta Musei Silesiae Scientiae Naturales**, v. 70, n. 1, p. 65–74, 2021.

MAPELI, N. C. *et al.* Deterrência alimentar em *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) induzida por soluções homeopáticas. **Revista Ceres**, v. 62, n. 2, p. 184–190, 2015.

MARSDEN, T.; SONNINO, R. Human health and wellbeing and the sustainability of urban-regional food systems. **Current Opinion in Environmental Sustainability**, v. 4, n. 4, p. 427–430, 2012.

MATSUBAYASHI, H. *et al*. Cytological mapping of ommutants of *Drosophila ananassae*. **Japanese Journal of Genetics**, v.67, p. 259-264, 1992.

MAZZETTO, F. *et al.* Monitoring of the exotic fly *Drosophila suzukii* in stone, pome and soft fruit orchards in NW Italy. **Journal of Asia-Pacific Entomology**, v. 18, n. 2, p. 321–329, 2015.

MAZZETTO, F. *et al.* Relationships between *Drosophila suzukii* and grapevine in North-Western Italy: Seasonal presence and cultivar susceptibility. **Bulletin of Insectology**, v. 73, n. 1, p. 29–38, 2020.

MELLO, L. M. R.; MACHADO, C. A. E. **Vitivinicultura brasileira: panorama 2019**. *In*: COMUNICADO TÉCNICO 214. Bento Gonçalves: Embrapa Uva e Vinho, 2020. p. 12.

MIELE, A.; MANDELLI, F. Manejo do dossel vegetativo e seu efeito nos componentes de produção da videira Merlot. **Revista Brasileira de Fruticultura**, v. 34, n. 4, p. 964–973, 2012.

Ministério da Agricultura, Pecuária e Abastecimento – MAPA. (). **Agrofit**: Sistemas de Agrotóxicos Fitossanitários, 2003. Avaliable at: http://agrofit.agricultura.gov.br /agrofit_cons/principal_agrofit_cons.

MITSUI, H.; BEPPU, K.; KIMURA, M. T. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. **Entomological Science**, v. 13, n. 1, p. 60–67, 2010.

MODOLON, T. A. *et al.* Diet and development of the fall armyworm *Sopodoptera frugiperda* Smith (Lepidoptera: Noctuidae) en maize treated with the homeopathic preparation Silicea terra. **Revista Brasileira de Agroecologia**, v. 12, n. 2, p. 106–116, 2017.

MODOLON, T. A. *et al.* Tomato Crop Under Organic Production System. **Horticultura Brasileira**, v. 30, n. 1, p. 51–57, 2012.

MOHAMED, H. A.; GAD, H. A.; ORABY, H. K. Field larvicidal and pupicidal efficacy of the natural insecticide, oxymatrine against *Culex* species in Beni Suef Governorate, Egypt. **Journal of Natural Pesticide Research**, v. 4, p. 100030, 2023.

MONTGOMERY, D. C.; RUNGER, G. C. **Estatística aplicada e probabilidade para engenheiros**. 5. ed. Rio de Janeiro: LTC, 2013.

MORAL, R. A.; HINDE, J.; DEMÉTRIO, C. G. B. Half-normal plots and overdispersed models in R: The hnp package. **Journal of Statistical Software**, v. 81, n. 10, 2017.

MORENO, N. M. Agrohomeopathy or agrochemicals?. **Allgemeine Homöopathische Zeitung**, 262, p. PR03, 2017.

NIJLAND, R.; HELSEN, H. Zoeken naar de zwakke plek van suzuki. **Wageningenworld**, v. 2, n. 65, p. 22–27, 2015.

NUNES, A. M., *et al*. Primeiros registros de *Drosophila suzukii* (Diptera: Drosophilidae) em agroecossistemas na metade sul do Rio Grande do Sul. *In*: XXV Congresso Brasileiro de Entomologia, 2014, Goiânia, GO. **Anais** [...] Goiania, GO: Universidade Federal de Goiás, 2014.

O'GRADY, P. M.; BEARDSLEY, J. W.; PERREIRA, W. D. New records for introduced Drosophilidae (Diptera) in Hawai'i. **Bishop Museum Occasional Papers**, v. 69, p. 34-35, 2002.

OLIVEIRA, A. S. de; AMARAL NETO, J. A. B. do; SANTOS, R. S. S. dos. Primeiro registro de *Drosophila suzukii* (Diptera: Drosophilidae) em pomar de macieira em Vacaria- Rio Grande do Sul. *In*: 5º Encontro de iniciação científica da embrapa uva e vinho e 1º encontro de pós-graduandos da embrapa uva e vinho. **Anais** [...] Bento Gonçalves: Embrapa Uva e Vinho, 2015.

ØRSTED, I. V.; ØRSTED, M. Species distribution models of the Spotted Wing Drosophila (*Drosophila suzukii*, Diptera: Drosophilidae) in its native and invasive range reveal an ecological niche shift. **Journal of Applied Ecology**, v. 56, n. 2, p. 423–435, 2019.

PADILHA, A. C. *et al.* Avaliação de atrativos alimentares na captura de Drosophila suzukii na cultura da videira. *In*: Anais XXVI Congresso Brasileiro de Entomologia; IX Congresso Latino-americano de EntomologiaI. **Anais** [...] Maceio: Sociedade Entomologica Brasileira, 2016.

PANDOLFO, C.; VIANNA, L. F. de N. **Caracterização da região produtora, indicadores e instrumentos para proposição de uma indicação geográfica**. 2020.

PANEL, A. D. C. *et al.* Overwintered drosophila suzukii are the main source for infestations of the first fruit crops of the season. **Insects**, v. 9, n. 4, 2018.

PANEL, A. D. C. **Seasonal biology of *Drosophila suzukii*: Genetic and phenotypic variation in the Netherlands**. 2021. 299 f. University of Groningen, 2021.

PANEL, A. D. C. *et al.* Seasonal morphotypes of *Drosophila suzukii* differ in key life-history traits during and after a prolonged period of cold exposure. **Ecology and Evolution**, v. 10, n. 17, p. 9085–9099, 2020.

PAPANASTASIOU, S. A. *et al.* Population dynamics of *Drosophila suzukii* in coastal and mainland sweet cherry orchards of Greece. **Insects**, v. 11, n. 9, p. 1–19, 2020.

PAULA, M. A.; LOPES, P. H. S.; TIDON, R. First record of *Drosophila suzukii* in the Brazilian Savanna. **Drosophila Information Service**, v. 97, p. 113-115, 2014.

PELTON, E.; GRATTON, C.; GUÉDOT, C. Susceptibility of cold hardy grapes to *Drosophila suzukii* (Diptera: Drosophilidae). **Journal of Applied Entomology**, v. 141, n. 8, p. 644–652, 2017.

POYET, M. *et al.* Invasive host for invasive pest: When the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. **Agricultural and Forest Entomology**, v. 16, n. 3, p. 251–259, 2014.

POYET, M. *et al.* The wide potential trophic niche of the asiatic fruit fly *Drosophila suzukii*: The key of its invasion success in temperate Europe?. **PLoS ONE**, v. 10, n. 11, 2015.

PROVOST, C.; PEDNEAULT, K. The organic vineyard as a balanced ecosystem: Improved organic grape management and impacts on wine quality. **Scientia Horticulturae**, v. 208, p. 43–56, 2016.

R CORE TEAM. **R: A language and environment for statistical computing**. Vienna, Austria: Foundation for Statistical Computing, 2021.

RAMANKUTTY, N. *et al.* Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. **Annual Review of Plant Biology**, v. 69, p. 789–815, 2018.

RICE, K. B.; SHORT, B. D.; LESKEY, T. C. Development of an attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae): Evaluation of attracticidal spheres under laboratory and field conditions. **Journal of Economic Entomology**, v. 110, n. 2, p. 535–542, 2017.

RIGBY, R. A.; STASINOPOULOS, D. M.; LANE, P. W. Generalized additive models for location, scale and shape. **Journal of the Royal Statistical Society. Series C: Applied Statistics**, v. 54, n. 3, p. 507–554, 2005.

ROSSI-STACCONI, M. V. *et al.* Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii*: useful clues for control strategies. **Journal of Pest Science**, v. 89, n. 3, p. 689–700, 2016.

ROUZÈS, R. *et al.* First occurence of *Drosophila suzukii* in the Sauternes vineyards. **Journal International des Sciences de la Vigne et du Vin**, v. 46, n. 2, p. 145–147, 2012.

RUPP, L. C. D. *et al.* High dilution of Staphysagria and fruit fly biotherapic preparations to manage South American fruit fly, *Anastrepha fraterculus*, in organic peach orchards. **Biological Agriculture and Horticulture**, v. 28, n. 1, p. 41–48, 2012.

SAGUEZ, J.; LASNIER, J.; VINCENT, C. First record of *Drosophila suzukii* in quebec vineyards. **Journal International des Sciences de la Vigne et du Vin**, v. 47, n. 1, p. 69–72, 2013.

SANTOIEMMA, G. *et al.* Habitat preference of *Drosophila suzukii* across heterogeneous landscapes. **Journal of Pest Science**, v. 92, n. 2, p. 485–494, 2019.

SANTOIEMMA, G. *et al.* Semi-natural habitats boost *Drosophila suzukii* populations and crop damage in sweet cherry. **Agriculture, Ecosystems and Environment**, v. 257, p. 152–158, 2018.

SANTOS, R. S. S. dos. *Drosophila suzukii* (Matsumura, 1931) (DIPTERA: DROSOPHILIDAE) atacando frutos de morangueiro no brasil. **Enciclopédia Biosfera**, v. 10, n. 18, p. 4005, 2014.

SANTOS, L. A. *et al.* Global potential distribution of *Drosophila suzukii* (Diptera, Drosophilidae). **PLoS ONE**, v. 12, n. 3, p. 1–13, 2017.

SCHETELIG, M. F. *et al.* Environmentally sustainable pest control options for *Drosophila suzukii.* **Journal of Applied Entomology**, v. 142, n. 1–2, p. 3–17, 2018.

SCHLESENER, D. C. H. *et al.* Biology and fertility life table of *Drosophila suzukii* on artificial diets. **Entomologia Experimentalis et Applicata**, v. 166, n. 11–12, p. 932–936, 2018.

SCHLESENER, D. C. H. *et al. Drosophila suzukii*: Nova Praga Para a Fruticultura Brasileira. **O Biologico**, 2015, p. 7.

SCHLESENER, D. C. H. *et al.* Insecticide Toxicity to *Drosophila suzukii* (Diptera: Drosophilidae) parasitoids: *Trichopria anastrephae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae). **Journal of Economic Entomology**, v. 112, n. 3, p. 1197–1206, 2019.

SCHÖNEBERG, T. *et al.* Pruning of small fruit crops can affect habitat suitability for *Drosophila suzukii*. **Agriculture, Ecosystems and Environment**, v. 294, p. 106860, 2020.

SEN, S. *et al.* Agrohomeopathy: an Emerging Field of Agriculture for Higher Crop Productivity and Protection of Plants Against Various Stress Conditions. **International Journal of Research and Analytical Reviews**, v. 5, n. 4, p. 52–56, 2018.

SHAWER, R. Chemical control of *Drosophila suzukii*. In: Garcia, F.R.M. (eds) ***Drosophila suzukii* Management**. Springer, 2020.

SHEARER, P. W. *et al.* Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. **BMC Ecology**, v. 16, n. 1, p. 1–18, 2016.

SHI, L. *et al.* Extratropical impacts on the 2020–2023 Triple-Dip La Niña event. **Atmospheric Research**, v. 294, p. 106937, 2023.
SHRADER, M. E.; BURRACK, H. J.; PFEIFFER, D. G. *Drosophila suzukii* (Diptera: Drosophilidae) Oviposition and Adult Emergence in Six Wine Grape Varieties Grown in Virginia. **Journal of Economic Entomology**, v. 112, n. 1, p. 139–148, 2019.

SILVA, G. F. da *et al.* Homeopathic Solutions as a Tool for the Agroecological Management of Velvetbean Caterpillar. **Brazilian Archives of Biology and Technology**, v. 66, p. e23220046, 2023.

Silveira-Neto, S. *et al*. **Fatores ecológicos: Manual de Ecologia dos Insetos**. Agronômica Ceres, Piracicaba, 1976.

SINN, F.; CONSULENZA, C. *Drosophila suzukii* – osservazioni nella tarda estate 2011. **Frutta e Vite**, v. 2, p. 49-52, 2012.

SMIRLE, M. J. *et al.* Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) populations from British Columbia, Canada. **Pest Management Science**, v. 73, n. 1, p. 130–137, 2017.

SOUZA, G. K. *et al. Acca sellowiana* (Myrtaceae): A New Alternative Host for *Drosophila suzukii* (Diptera: Drosophilidae) in Brazil. **Florida Entomologist**, v. 100, n. 1, p. 190–191, 2017.

STEPHENS, A. R. *et al.* Cold Hardiness of Winter-Acclimated *Drosophila suzukii* (Diptera: Drosophilidae) Adults. **Environmental Entomology**, v. 44, n. 6, p. 1619–1626, 2015.

STOCKTON, D. *et al.* Interactions between biotic and abiotic factors affect survival in overwintering *Drosophila suzukii* (Diptera: Drosophilidae). **Environmental Entomology**, v. 48, n. 2, p. 454–464, 2019.

TAIT, G. *et al. Drosophila suzukii* (Diptera: Drosophilidae): A Decade of Research towards a Sustainable Integrated Pest Management Program. **Journal of Economic Entomology**, v. 114, n. 5, p. 1950–1974, 2021.

TAIT, G. *et al.* **Noncrop Host Plants Used By Spotted-wing Drosophila**. Oregon State University Extension Service, 2019.

THERNEAU, T. **A Package for Survival Analysis in R**. R package version 3.5-5, 2023.

TOCHEN, S. *et al.* Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. **Journal of Applied Entomology**, v. 140, n. 1–2, p. 47–57, 2016.

TOCHEN, S. *et al.* Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. **Environmental Entomology**, v. 43, n. 2, p. 501–510, 2014.

TONIETTO, J.; SOTÉS RUIZ, V.; GÓMEZ-MIGUEL, V. **Clima, Zonificación y Tipicidad del Vino en Regiones Vitivinícolas Iberoamericanas**. Madrid: CYTED, 2012.

TONINA, L. *et al.* Texture features explain the susceptibility of grapevine cultivars to *Drosophila suzukii* (Diptera: Drosophilidae) infestation in ripening and drying grapes. **Scientific Reports**, v. 10, n. 1, p. 1–13, 2020.

TSCHARNTKE, T. *et al.* Conservation biological control and enemy diversity on a landscape scale. **Biological Control**, v. 43, n. 3, p. 294–309, 2007.

UMAR, A. M.; AISAMI, A. Acetylcholinesterase Enzyme (AChE) as a Biosensor and Biomarker for Pesticides: A Mini Review. **Bulletin of Environmental Science and Sustainable Management**, v. 4, n. 1, p. 7–12, 2020.

URBANEJA-BERNAT, P. *et al.* Non-crop habitats serve as a potential source of spotted-wing drosophila (Diptera: Drosophilidae) to adjacent cultivated highbush blueberries (Ericaceae). **Canadian Entomologist**, v. 152, n. 4, p. 474–489, 2020.

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

VAN TIMMEREN, S.; ISAACS, R. *Drosophila suzukii* in Michigan vineyards, and the first report of *Zaprionus indianus* from this region. **Journal of Applied Entomology**, v. 138, n. 7, p. 519–527, 2014.

VENABLES, W. N.; RIPLEY, B. D. **Modern Applied Statistics with S**. 4. ed. New York: Springer, 2002.

VERDI, R. *et al.* Homeopathic Management in Irrigated Rice Crop. **Brazilian Journal of Development**, v. 6, n. 9, p. 65540–65549, 2020.

VIANNA, L. F. *et al.* Caracterização agronômica e edafoclimática dos vinhedos de elevada altitude. **Revista de Ciências Agroveterinárias**, v. 15, n. 3, p. 215–226, 2016.

VIEIRA, J. G. A. *et al.* Some aspects of the biology of trichopria anastrephae (Hymenoptera: Diapriidae), a resident parasitoid attacking *Drosophila suzukii* (Diptera: Drosophilidae) in Brazil. **Journal of Economic Entomology**, v. 113, n. 1, p. 81–87, 2020.

VIJNOVSKY, B. **Tratado de Materia Medica HomeopaticaCA**. Rio de Janeiro, 1980.

VILELA, C. R.; MORI, L. The invasive spotted-wing Drosophila (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil). **Revista Brasileira de Entomologia**, v. 58, n. 4, p. 371–375, 2014.

VLACH, J. Identifying *Drosophila suzukii*. **Oregon Department of Agriculture**, p. 1–6, 2010.

VU, V. Q. **ggbiplot: A ggplot2 based biplot**. R package version 0.55, 2011.

WALSH, D. B. *et al. Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. **Journal of Integrated Pest Management**, v. 2, n. 1, p. 3–9, 2011.

WANG, X.; KAÇAR, G.; DAANE, K. M. Temporal dynamics of host use by *Drosophila suzukii* in California's San Joaquin valley: Implications for area-wide pest management. **Insects**, v. 10, n. 7, 2019.

WEISSINGER, L. *et al.* Effects of variety and grape berry condition of *Vitis vinifera* on preference behavior and performance of *Drosophila suzukii*. **Insects**, v. 10, n. 12, p. 1–15, 2019.

WEISSINGER, L. *et al.* Influences of blackberry margins on population dynamics of *Drosophila suzukii* and grape infestation in adjacent vineyards. **Journal of Applied Entomology**, v. 143, n. 8, p. 802–812, 2019.

WICKHAM, H. **ggplot2: Elegant Graphics for Data Analysis**. Springer-Verlag, New York, 2016.

WIMAN, N. G. *et al.* Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. **PLoS ONE**, v. 9, n. 9, 2014.

WISE, J. C. *et al.* IR-4 Attract N Kill Tactics for Control of Spotted Wing Drosophila in Blueberry, 2017. **Arthropod Management Tests**, v. 43, n. 1, p. 1–2, 2018.

WOLLMANN, J. *et al.* Infestation index of *Drosophila suzukii* (Diptera: Drosophilidae) in small fruit in southern Brazil. **Arquivos do Instituto Biológico**, v. 87, p. 1–9, 2020.

WOLLMANN, J. *et al.* Population Dynamics of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops in Southern Brazil. **Neotropical Entomology**, v. 48, n. 4, p. 699–705, 2019.

WYSS, E. *et al.* Homeopathic preparations to control the rosy apple aphid (Dysaphis plantaginea Pass.). **The Scientific World Journal**, v. 10, p. 38–48, 2010.

XU, J. *et al.* Evaluation of biological activities, and exploration on mechanism of action of matrine–cholesterol derivatives. **Bioorganic Chemistry**, v. 94, p. 103439, 2020.

YEH, D. A. *et al.* The Economic Impacts and Management of Spotted Wing Drosophila (*Drosophila suzukii*): The Case of Wild Blueberries in Maine. **Journal of Economic Entomology**, v. 113, n. 3, p. 1262–1269, 2020.

YUZUKI, K.; TIDON, R. Identification key for drosophilid species (Diptera, Drosophilidae) exotic to the Neotropical Region and occurring in Brazil. **Revista Brasileira de Entomologia**, v. 64, n. 1, p. 1–9, 2020.

ZANARDI, O. Z. *et al.* Bioactivity of a matrine-based biopesticide against four pest species of agricultural importance. **Crop Protection**, v. 67, p. 160–167, 2015.

ZANUNCIO-JUNIOR, J. S. *et al.* Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in Southeastern Brazil. **Florida Entomologist**, v. 101, n. 3, p. 522–525, 2018.