Universidade de São Paulo
Escola Superior de Agricultura "Luiz de Queiroz"

Dynamics of competition for resources in *Drosophila suzukii* (Diptera: Drosophilidae), *Zaprionus indianus* (Diptera: Drosophilidae) and entomopathogenic fungi

Larine de Paiva Mendonça

Tese apresentada para obtenção do título Doutora em Ciências. Área de concentração: Entomologia

Piracicaba
2024

Larine de Paiva Mendonça
Engenheira Agrônoma

Dynamics of competition for resources in *Drosophila suzukii* (Diptera: Drosophilidae), *Zaprionus indianus* (Diptera: Drosophilidae) and entomopathogenic fungi

Orientador:
Prof. **Dr. WESLEY AUGUSTO CONDE GODOY**

Tese apresentada para obtenção do título Doutora em Ciências. Área de concentração: Entomologia

Piracicaba
2024

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Mendonça, Larine de Paiva

Dynamics of competition for resources in *Drosophila suzukii* (Diptera: Drosophilidae), *Zaprionus indianus* (Diptera: Drosophilidae) and entomopathogenic fungi / Larine de Paiva Mendonça - - Piracicaba, 2024.

68 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Ecology 2. Interactions 3. Competition 4. Invasive pests I. Título

DEDICATION

Dedico esta tese ao meu pai, minha mãe e minha irmã, por serem meu suporte e sempre me incentivarem.

ACKNOWLEDGEMENTS

To God for the possibility to be here, for always being by my side, and for giving me strength in the hard times.
To Nossa Senhora do Carmo for the protection and love.
To my family, particularly my parents Clécio e Marilda for all suport, love and for never giving up on me. Thank you for being my example, and teaching me all the good values. I love you with all my heart.
To my sister, Lícia, even with the distance, keeps being my best friend, always believing in me.
To Guilherme for all the love and emotional support helping me to do not give up on the hard times.
To Wesley, my adviser, not only for the opportunity and for the new knowledge shared, but for being a model of leadership and emotional intelligence that I will always remember and carry with me in my heart and memories forever. Thanks for the friendship, all the advice and for always finding a way to make us feel better regardless of the chaos.
To Khalid, my co-adviser since 2013 for all the shared knowledge, support and help.
To the University of São Paulo – USP- ESALQ for the welcome and the opportunity to take my Ph.D to develop something that I love so much, research with entomolgy.
To the Entomology Postgraduate Program and the Department of Entomology and Acarology at Esalq/USP for the opportunity to take the doctoral course and for the infrastructure offered. To the teachers for the shared knowledge.
To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing the financial support.
To João Gorá, who, as a laboratory technician, not only offered support to our research but also wonderful stories and friendship.
To the Forest Ecology and Entomology Laboratory members, Igor, Isabela, Maiara, Maisa, Rayana, Walter, Anna Mara, Lucas for all the support, ideas, coffees, and friendship shared.
To the Laboratory of Pathology and Microbial Control (ESALQ/USP) and Prof. Dr. Italo Delalibera Junior for sharing equipment, for providing their structure, materials, and knowledge.
To the Agricultural Acarology Laboratory for providing the mites used in the tests in this thesis.
To all the friends that I made in Piracicaba, you were my family there and were present in the happy and hard times.

SUMMARY

RESUMO

Dinâmica de competição por recursos em *Drosophila suzukii* (Diptera: Drosophilidae), *Zaprionus indianus* (Diptera: Drosophilidae) e fungos entomopatogênicos

A chegada de pragas invasoras nos últimos tempos tem sido facilitada devido aos avanços da globalização. É considerada uma espécie invasora os indivíduos que chegam e permanecem em um novo local, diferente do seu de origem. Desta forma, *Drosophila suzukii* e *Zaprionus indianus* são consideradas pragas invasoras que chegaram recentemente ao Brasil. Tanto a *D. suzukii* como *Z. indianus* são pragas polífagas, ou seja, utilizam diversos frutos como fonte de alimentação, possuindo desta forma vários substratos em comum. Por meio do compartilhamento do alimento e do espaço para desenvolvimento dos seus indivíduos as espécies estão submetidas a diferentes tipos de interação como a interação competitiva. A competição é um processo natural que pode ocorrer de diversas formas como: competição intraespecífica, interespecífica e competição aparente. A competição intraespecífica ocorre entre indivíduos da mesma espécie, a interespecífica entre indivíduos de espécies diferentes e a competição aparente é uma interação indireta entre indivíduos que compartilham um inimigo natural em comum. Desta forma, investigamos os efeitos das diferentes interações competitivas entre as espécies invasoras e o seu efeito em parâmetros biológicos, comportamentais e seu efeito no controle destas pragas. Primeiramente confirmamos a coocorrência das espécies invasoras em campo. Em ensaios de laboratório exploramos o efeito da competição intra e interespecífica no desenvolvimento, reprodução, comportamento e projeção no tempo dessa interação. A *D. suzukii*, uma praga primária, não demonstrou ser afetada pela presença da sua competidora enquanto *Z. indianus* mostrou taxas reprodutivas elevadas quando na presença da *D. suzukii*, refletindo as densidades encontradas a campo. O efeito da densidade intraespecífica no controle biológico devido a interação indireta de fungos entomopatogenicos endofíticos na cultura do morango também foi explorado. A interação foi investigada para pragas de diferentes partes da planta (praga foliar e praga dos frutos) em duas cultivares de morango (Camarosa e San Andreas) tratadas com dois fungos entomopatogenicos diferentes (*Beauveria bassiana* e *Metarhizium robertsii).* A capacidade de controle demonstrou ser dependente da cultivar e da densidade da praga uma vez que foram observados uma sobrecompensação para os tratamentos que envolviam a combinação dos dois estresses: inoculação fúngica e alta densidade. Desta forma, mostrou-se que a eficiência do controle depende do monitoramento das densidades das pragas em campo, da cultivar usada e mais processos de inoculação devem ser feitos para garantir a capacidade de controle dessa técnica. Por fim foi avaliado a existência de competição aparente entre as pragas coocorrentes *D. suzukii* e *Z. indianus* compartilhando o inimigo natural *Metarhizium spp.* Embora a interação direta entre o fungo e as pragas individualmente tenham demonstrado promissoras para o controle, não foi possível observar fortes evidências de competição aparente ocorrendo quando as pragas estão interagindo por meio do inimigo natural. Os resultados destacam a importância do estudo das interações competitivas para melhor entendimento do desenvolvimento da praga como na decisão das melhores formas de controle das mesmas.

Palavras-chave: Ecologia, Interações, Competição, Pragas invasoras

ABSTRACT

Dynamics of competition for resources in *Drosophila suzukii* (Diptera: Drosophilidae), *Zaprionus indianus* (Diptera: Drosophilidae) and entomopathogenic fungi.

The arrival of invasive pests in recent years has been facilitated due to advances in globalization. Species are considered invasive when individuals arrive and remain in a new location different from their original location. Therefore, *Drosophila suzukii* and *Zaprionus indianus* are considered invasive pests that recently arrived in Brazil. Both *D. suzukii* and *Z. indianus* are polyphagous pests. Therefore, they use different fruits as a food source, thus having several substrates in common. By sharing food and space for the development of their individuals, the species are subjected to different types of interaction, such as competitive interaction. Competition is a natural process that can occur in different ways, such as intraspecific, interspecific, and apparent competition. Intraspecific competition involves individuals of the same species exploiting the same resources. Interspecific competition occurs between individuals of different species, and apparent competition is an indirect interaction between individuals sharing a common natural enemy. In this way, we investigated the effects of different competitive interactions between invasive species and their effect on biological and behavioral parameters and their effect on the control of these pests. Firstly, we confirmed the co-occurrence of invasive species in the field. In laboratory tests, we explored the effect of intra and interspecific competition on development, reproduction, behavior, and projection of this interaction over time. *D. suzukii*, a primary pest, was not affected by the presence of its competitor while *Z. indianus* showed high reproductive rates when in the presence of *D. suzukii,* reflecting the densities found in the field. The effect of intraspecific density on the biological control due the indirect interaction of endophytic entomopathogenic fungi in strawberry crops was also explored. The interaction was investigated for pests from different parts of the plant (foliar parts and fruit parts) in two strawberry cultivars (Camarosa and San Andreas) treated with two different entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium robertsii).* The control capacity proved to be dependent on the cultivar and pest density since overcompensation was observed for treatments involving the combination of two stresses: fungal inoculation and high density. This shows that control efficiency depends on monitoring pest densities in the field, the cultivar used and the frequency of inoculation processes that must be carried out to guarantee the control capacity of this technique. Finally, the existence of apparent competition between the co-occurring pests *D. suzukii* and *Z. indianus* sharing the natural enemy *Metarhizium spp* was assessed. Although direct interactions between the fungus and individual pests have shown promise for control, it has not been possible to observe strong evidence of apparent competition occurring when pests are interacting with the natural enemy. The results highlight the importance of studying competitive interactions to better understand the pest development and to evaluate the best ways to control them.

Keywords: Ecology, Interactions, Competition, Invasive pests

1 GENERAL INTRODUCTION

Globalization is a process that facilitates the movement of people, and consequently, invasive insects once they arrive in regions far from their origin and can cause significant economic losses (OLSON, 2006). With the evolution of globalization at an accelerated pace, the possibilities of invasion pests grow in the same proportion, facilitating the movement of species to areas around the world previously unbeknownst to them (VENETTE and HUTCHISON, 2021). Invasion is characterized by the process where individuals colonize and persist in a location or locations distant from their origin (SHIGESADA and KAWASAKI, 1997; WILLIAMSON and GRIFFITHS, 1996). Among the current invasive pests that arrived in Brazil, we highlight *Zaprionus indianus and Drosophila suzukii* (DEPRÁ *et al.*, 2014; VILELA, 1999). The *Z. indianus* was first reported in Brazil in 1999 in the state of São Paulo (VILELA, 1999) and the spotted-wig drosophila, *D. suzukii* was first noticed in 2012/2013 in the south region of Brazil (DEPRÁ *et al.*, 2014).

Drosophila suzukii, unlike other drosophilids, can be a pest, ovipositing in thin-shelled fruits due to a modification in the female ovipositor, with a serrated characteristic (MITSUI; TAKAHASHI; KIMURA, 2006). *Zaprionus inidianus* is considered a secondary pest because the fruit needs to be damaged to establish itself, a primary pest only in fig plantations (LINDE *et al.*, 2006; VILELA and GOÑI, 2015). The simultaneous presence of these two pests has already been reported in the literature, causing damage to fruit crops such as strawberries (BERNARDI *et al.*, 2015; LASA and TADEO, 2015; MENDONCA *et al.*, 2019). The establishment of invasive species in the new place depends on different factors. Among the determining factors for the establishment of invasive pests is the presence of food. However, other factors can also affect such as adaptation to the region's climate and the absence of natural enemies are important, but the impact of competitors on the invasive species has been underestimated (CRAWLEY *et al.*, 1986).

Competition is a natural process and can be of the intraspecific type, with individuals of the same species interacting. The interspecific type is characterized by individuals of different species competing for the same resource, territory, or partner. Apparent competition, on the other hand, is a negative indirect interaction between individuals sharing a natural enemy (HOLT, 1977; TILMAN, 1982; YODZIS, 2013). For the competition to happen the individual needs to be in contact with another individual, directly or indirectly (BIRCH, 1957). In the case of intraspecific and interspecific competition, individuals share the same resource, space, or sexual partner (TILMAN, 1982; YODZIS, 2013). For apparent competition to occur, the individuals do not necessarily need to share resources, but they are expected to share the natural enemy (HOLT, 1977). Apparent competition, although it may seem like a mere interpretation of ecological scenarios of trophic interactions, has high relevance in biological control scenarios, opening a new perspective for evaluating the effectiveness of using certain polyphagous natural enemies in pest control (LANGER and HANCE, 2004).

Drosophila suzukii and *Z. indianus* are polyphagous organisms, feeding on different food sources (KIRSCHBAUM, 2020; VILELA, 1999). In the case of these species, they share the host fruits on which they feed and also the space for development and pupate (BERNARDI *et al.*, 2017; KENIS *et al.,* 2016; MENDONCA *et al.*, 2019; PREZOTO and BRAGA, 2013; ZANUNCIO-JUNIOR *et al.*, 2018). In addition to the food source and space, *D. suzukii* and *Z. indianus* may also have natural enemies in common (ESTECA, 2021; MARCHIORI, 2003; STACCONI *et al,* 2013; WOLTZ and LEE, 2017). Among the natural enemies these pests have in common, we highlight entomopathogenic fungi (IBOUH *et al.,* 2019; SVEDESE *et al.*, 2012). Fungi are microscopic organisms that occur naturally in the environment and can act as decomposers of organic matter and biological control agents (DEACON, 2013).

Entomopathogenic fungi have attracted the attention of strategic sectors in the agricultural sciences scenario mainly because they are an alternative form to chemical control, which in turn has demonstrated problems over time, with impacts on the environment, insect resurgence, and pest resistance (CHARNLEY and COLLINS, 2007). Furthermore, in a single genus (for example *Beauveria spp.*), it is possible to verify a high rate of infection over a large diversity of insect species, guaranteeing success in the control of several agricultural pests (GOETTEL, 1990).

The interaction between the entomopathogenic fungus and the pests *D. suzukii* and *Z. indianus* has been extensively explored in the literature through the direct interaction of these individuals (COSSENTINE, ROBERTSON, BUITENHUIS, 2016; SVEDESE *et al.,* 2012). However, few studies have addressed the indirect interaction between them. The indirect interaction consists of interaction mediated by another individual (WOOTTON, 1994). Among the indirect interactions, we can highlight apparent competition and endophytic interaction. Few studies have investigated apparent competition involving entomopathogenic fungi (MEYLING and HAJEK, 2010). Most studies focused on apparent competition have been proposed to analyze predators and parasitoids (MORRIS; LEWIS; GODFRAY, 2004; MULLER and GODFRAY, 1997). The endophytic relationship is a symbiotic interaction between the plant and the fungus, where the fungus grows within the plant's tissues, establishing a reciprocal nutrient exchange relationship (MOONJELY; BARELLI; BIDOCHKA, 2016). The indirect interaction is established by the fungus by the induction of the defenses of the plant against the attack of the pests (CHANETON and OMACINI, 2007). The endophytic interaction has demonstrated potential for biological pest control (CANASSA *et al.*, 2020; MWAMBURI, 2021), although the effect of endophytic interaction on pests with high densities has not been explored.

Therefore, in order to explore this interaction between individuals, the objectives of this work were: (i) to evaluate the effect in the laboratory of intra and interspecific competition between *D. suzukii* and *Z. indianus* on biological parameters and its effect on population growth throughout of time (ii) evaluate the endophytic effect of the treatment of strawberry plants with entomopathogenic fungi on pests of foliar and pest of fruits part when under conditions of intraspecific competition and (iii) evaluate the effect of apparent competition between co-occurring pests *D. suzukii* and *Z. indianus* with entomopathogenic fungi. Thus, assuming that there is a pressure of competition between the invasive species, the hypotheses tested were: (i) the variation in the values of the biological parameters of the two invasive species under competition will produce different ecological patterns of population fluctuation over time, characterized by regular, irregular periodic oscillations or stable equilibrium, (ii) there will be an endophytic effect of the treatment of strawberry plants with entomopathogenic fungi on pests of foliar and fruit parts under competitive conditions, affecting the choice of the plant and the development of individuals, (iii) the immature phase of *D. suzukii* and *Z. indianus*, when present in the soil, will be subject to the direct effect of the entomopathogenic fungus, thus becoming dependent on the indirect effect of apparent competition.

References

1. BERNARDI D. *et al.* Susceptibility and interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera : Drosophilidae) in damaging strawberry. **Neotropical Entomology**, *[S. l.]*, v. 46, n. 1, p. 1–7, 2017. DOI: 10.1007/s13744-016-0423-9.
2. BERNARDI D. *et al.* Duplo ataque. **Embrapa Uva e Vinho-Artigo em periódico indexado (ALICE)**, *[S. l.]*, 2015.
3. BIRCH I. C. The meaning of competition. **The american naturalist**, *[S. l.]*, v. 91, p. 5–18, 1957.

4. CANASSA F. *et al.* Fungal isolate and crop cultivar influence the beneficial effects of root inoculation with entomopathogenic fungi in strawberry. **Pest Management Science**, *[S. l.]*, v. 76, n. 4, p. 1472–1482, 2020. DOI: 10.1002/ps.5662.
5. CHANETON E. J.; OMACINI, M. Bottom-up cascades induced by fungal endophytes in multitrophic systems. **Ecological communities: plant mediation in indirect interaction webs**, *[S. l.]*, p. 164–187, 2007.
6. CHARNLEY A. K.; COLLINS S. A. 10 Entomopathogenic Fungi and Their Role in Pest Control. **Environmental and microbial relationships**, *[S. l.]*, v. 4, p. 159, 2007.
7. COSSENTINE J.; ROBERTSON M.; BUITENHUIS R. Impact of acquired entomopathogenic fungi on adult *Drosophila suzukii* survival and fecundity. **Biological Control**, *[S. l.]*, v. 103, p. 129–137, 2016.
8. CRAWLEY M. J. *et al.* The Population Biology of Invaders. **Biological Sciences**, *[S. l.]*, v. 314, p. 711–731, 1986. DOI: 10.1098/rstb.1986.0082.
9. DEACON J. W. **Fungal biology**. [s.l: s.n.].
10. DEPRÁ M. *et al.* The first records of the invasive pest *Drosophila suzukii* in the South American continent. **Journal of Pest Science**, *[S. l.]*, v. 87, n. 3, p. 379–383, 2014. DOI: 10.1007/s10340-014-0591-5.
11. ESTECA F. C. **Environmental management strategies for pest control in strawberry crop**. 2021. *[S. l.]*, 2021.
12. GOETTEL M.S. *et al.* Safety to nontarget invertebrates of fungal biocontrol agents. *[S. l.]*, 1990.
13. HOLT D. R. Predation, apparent competition and the structure of prey communities. **Theoretical population biology**, *[S. l.]*, v. 229, p. 197–229, 1977.
14. IBOUH K. *et al.* Biological control of *Drosophila suzukii*: Efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. **Crop Protection**, *[S. l.]*, v. 125, n. July, p. 104897, 2019. DOI: 10.1016/j.cropro.2019.104897.
15. KENIS M. *et al.* Non-crop plants used as hosts by *Drosophila suzukii* in Europe. **Journal of Pest Science**, *[S. l.]*, v. 89, p. 735–748, 2016.
16. KIRSCHBAUM D.S. *et al.* The biology and ecology of *Drosophila suzukii* (Diptera: Drosophilidae). *In*: **Drosophila suzukii management. Springer**. [s.l: s.n.]. p. 41–91. DOI: doi.org/10.1007/978-3-030-62692-1_4.
17. LANGER A. ; HANCE T. Enhancing parasitism of wheat aphids through apparent competition: a tool for biological control. **Agriculture, ecosystems & environment**, *[S. l.]*, v. 102, n. 2, p. 205–212, 2004.
18. LASA R.; TADEO E. Invasive Drosophilid Pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. **Florida Entomologist**, *[S. l.]*, v. 98, n. 3, p. 987–988, 2015.
19. VAN DER LINDE K. *et al.* First records of *Zaprionus indianus* (Diptera : Drosophilidae), a pest species on commercial fruits from Panama and the United States of America **Florida Entomologist**, *[S. l.]*, v.89, n.3, p. 402-404, 2006.
20. MARCHIORI C. H. First report of parasitoid *Pachycrepoideus vindemiae* Rondani (Hymenoptera: Pteromalidae) in pupae of *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in Brazil. **Biotemas**, *[S. l.]*, p. 129–133, 2003.
21. MENDONCA L. D. P. *et al.* Host Potential and Adaptive Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Barbados Cherries. **Journal of Economic Entomology**, *[S. l.]*, v. 112, n. 6, p. 3002–3006, 2019. DOI: 10.1093/jee/toz195.
22. MEYLING N. V.; HAJEK A. E. Principles from community and metapopulation ecology: Application to

fungal entomopathogens. **BioControl**, *[S. l.]*, v. 55, n. 1, p. 39–54, 2010. DOI: 10.1007/s10526-009-9246-5.

23. MITSUI H.; TAKAHASHI K. H.; KIMURA M. T. Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. **Population Ecology**, *[S. l.]*, v. 48, p. 233–237, 2006. DOI: 10.1007/s10144-006-0260-5.
24. MOONJELY S.; BARELLI L.; BIDOCHKA M. J. Insect pathogenic fungi as endophytes. *In*: **Genetics and Molecular Biology of Entomopathogenic Fungi**. [s.l: s.n.], v.94, p. 107–135, 2016
25. MORRIS R. J.; LEWIS O. T.; GODFRAY, H. C. J. Experimental evidence for apparent competition in a tropical forest food web. **Nature**, *[S. l.]*, v. 428, n. 6980, p. 310–313, 2004. DOI: 10.1038/nature02394.
26. MULLER C. B.; GODFRAY H. C. J. Apparent Competition between Two Aphid Species. **The Journal of Animal Ecology**, *[S. l.]*, v. 66, n. 1, p. 57, 1997. DOI: 10.2307/5964.
27. MWAMBURI L. A. Endophytic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, confer control of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in two tomato varieties. **Egyptian Journal of Biological Pest Control**, *[S. l.]*, v. 31, n. 1, 2021. DOI: 10.1186/s41938-020-00357-3.
28. OLSON L J. The Economics of Terrestrial Invasive Species : A Review of the Literature. *[S. l.]*, v. 1, n. April, p. 178–194, 2006.
29. PREZOTO F.; BRAGA N. Predation of *Zaprionus indianus* (Diptera: Drosophilidae) by the social wasp *Synoeca cyanea* (Hymenoptera: Vespidae). **Florida Entomologist**, *[S. l.]*, v. 96, p. 670–672, 2013.
30. SHIGESADA N.; KAWASAKI, K. **Biological invasions: theory and practice.** [s.l: s.n.].
31. STACCONI M. V. R. *et al.* First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. **Entomologia**, *[S. l.]*, 2013.
32. SVEDESE V. M. *et al.* Ação de fungos entomopatogênicos em larvas e adultos da mosca do figo *Zaprionus indianus* (Diptera: Drosophilidae). **Ciencia Rural**, *[S. l.]*, v. 42, n. 11, p. 1916–1922, 2012. DOI: 10.1590/S0103-84782012001100002.
33. TILMAN D. **Resource competition and community structure.** [s.l: s.n.].
34. VENETTE R.C.; HUTCHISON W. D. Invasive insect species: Global challenges, strategies and opportunities. **Frontiers in Insect Science**, *[S. l.]*, v. 1, 2021.
35. VILELA C. R.; GOÑI B. Mosca-africana-do-figo, *Zaprionus indianus* Gupta. **Pragas introduzidas no Brasil: insetos e ácaros,** *[S. l.]*, 2015.
36. VILELA C. R. Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the neotropical region? **Drosophila Information Service**, *[S. l.]*, v. 82, p. 37–39, 1999.
37. WILLIAMSON M.; GRIFFITHS B. **Biological invasions**. [s.l.] : Springer Science & Business Media, 1996.
38. WOLTZ J. M.; LEE J. C. Pupation behavior and larval and pupal biocontrol of *Drosophila suzukii* in the field. **Biological Control**, *[S. l.]*, v. 110, n. March, p. 62–69, 2017. DOI: 10.1016/j.biocontrol.2017.04.007.
39. WOOTTON J. T. The nature and consequence of indirect effects in ecological communities. **Annual Review of Ecology and Systematics**, *[S. l.]*, v. 25, p. 443–466, 1994.
40. YODZIS P. **Competition for space and the structure of ecological communities.** [s.l: s.n.].
41. ZANUNCIO-JUNIOR, J. S. *et al.* Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in Southeastern Brazil. **Florida Entomologist**, *[S. l.]*, v. 101, n. 3, p. 522–525, 2018. DOI: 10.1653/024.101.0328.

2 EFFECTS OF CO-OCCURENCE AND INTRA- AND INTERSPECIFIC INTERACTIONS BETWEEN *Drosophila suzukii* AND *Zaprionus indianus*

This chapter has been published in: MENDONÇA, LDP; HADDI, K; GODOY, WAC (2023) Effects of co-occurrence and intra-and interspecific interactions between *Drosophila suzukii* and *Zaprionus indianus*. *PloS one*, v. 18, n. 3, p. e0281806. https://doi.org/10.1371/journal.pone.0281806 as a requirement for Ph.D students of the Graduate Program in Entomology, ESALQ-USP.

Abstract

In drosophilids, competition and coexistence can impact survivorship, growth, and reproductive output. Here, we evaluated direct competition between two co-occurring fruit flies, the spotted-wing drosophila *Drosophila suzukii* and the African fig fly *Zaprionus indianus*, comparing results from field collections with laboratory experiments. Field collections were conducted to evaluate co-occurrence between species. In the laboratory, different densities of eggs of each species were provided an artificial diet, and intra- and interspecific densities were evaluated regarding biological traits such as development and fecundity. Field collections showed a prevalence of *Z. indianus*, followed by other drosophilid species, including *D. suzukii*. Pupal survival and adult emergence were higher in *D. suzukii* than in *Z. indianus* at both intra- and interspecific densities, with decreasing values in response to increased densities. Fecundity did not differ significantly for either species at different intraspecific densities, but when reared together at different densities, *Z. indianus* was significantly more fecund than *D. suzukii*. Development time showed no significant difference at intraspecific densities, but when reared together, *Z. indianus* had longer development times than *D. suzukii*. Leslie Matrix projections indicated that *D. suzukii* showed practically the same dynamics at intraspecific and interspecific densities, with increasing oscillations at low and intermediate densities and decreasing oscillations at high densities. *Zaprionus indianus* showed a similar oscillation to *D. suzukii*, except at intermediate intraspecific densities, when the pattern was cyclic. Low interspecific densities resulted in decreasing oscillations. In the two-choice oviposition bioassays, *D. suzukii* females showed no significant preference for diets previously infested or not with either conspecific or heterospecific eggs at different densities. Understanding competitive interactions between co-occurring heterospecific species should be considered when establishing management tactics for spotted-wing drosophila.

Keywords: Invasive pests, Intraspecific competition, Interspecific competition *Drosophila suzukii* and *Zaprionus indianus*

2.1 Introduction

Two drosophilid species that damage small fruit have invaded the Neotropics in recent decades. The first invader, the African fig fly *Zaprionus indianus* Gupta (Diptera: Drosophilidae), an exotic drosophilid originating from sub-Saharan Africa [1] that has extended its range from tropical to temperate areas, and showing excellent plasticity to survive in environments with adverse conditions [2]. This fly was reported in Brazil in 1999 in the state of São Paulo [3] and has since spread throughout the country [4–6]. The second invasive drosophilid to arrive in Brazil was the spotted-wing drosophila *Drosophila suzukii* Matsumura, in 2012–2013 [7]. This species is of Asian origin but gained notoriety as an invasive pest of soft fruits with its spread around the world [8, 9]; it was first identified outside Asia in the United States and Europe around the year 2008 [10, 11]. Although reported to occur jointly, these two drosophilids present distinct biological and behavioral traits as well as seasonal phenotypic plasticity that influence their abilities to invade new areas and allow adaptations to different environments under a wide range of temperatures [12]. Indeed, females of *Z. indianus* produce around 70 eggs, can pause ovarian development during cold periods without loss of fertility [2, 13], and complete 12 to 16 generations per year. Dissimilarly, *Drosophila suzukii* females are highly fertile and lay more than 200 eggs during their lifetime [14]. They exhibit widely varying longevity (i.e., 35 days at 10°C and 2 days at 30°C), complete 3 to 10 generations per year, and are able to maintain relatively constant fecundity and longevity under low temperatures when afforded a short period of warm temperatures [15, 16]. Furthermore, *D. suzukii* has a high dispersal capacity, and depending on microclimatic factors can travel up to 100 m per day [17, 18].

In multiple invasions, the first invading species may gain a clear benefit in terms of abundance compared to subsequent invaders, as it will have more time to adapt to existing resources and challenges in the new land [19]. However, any advantage of the first colonizers will depend on their biological characteristics and their ability to use a wide range of resources, as well as the competitive stress from later-arriving species [20]. In this context, the interplay between the co-occurring *D. suzukii* and *Z. indianus* merits analysis because of their different biological traits and life strategies [21]. This interplay raises the question of which is the better invasion strategy. To colonize environments with damaged fruits first or to infest undamaged fruits first?

Drosophila suzukii is a polyphagous pest attacking a wide range of soft and thin-skinned fruits, which may have facilitated its establishment in different regions [22–24]. Differently from other drosophilids, *D. suzukii* damages the surface of fruits with its modified serrated ovipositor [9, 22]. This serrated ovipositor is believed to give *D. suzukii* an advantage over other drosophilids such as *Z. indianus*, as it allows the spotted-wing drosophila to use healthy fruits that were not previously used by heterospecific competitors [10, 19]. In contrast, *Z. indianus* is a secondary pest, able to infest only already-damaged fruits [1]. Despite its difficulty in ovipositing on healthy fruits, the importance of this pest increases when it occurs together with *D. suzukii*, since it can use the oviposition sites of *D. suzukii* as a gateway for its offspring, overcoming the previous advantage of the latter [25].

Independently of which species is the initial colonizer and whether it colonizes unhealthy or healthy fruits first, both invasive species must initially adjust to the positive or negative effects of low densities upon arrival and possible adverse conditions in the new location before attaining high densities [26]. For invasive species and since biological invasions start with low densities, low densities may be much more beneficial than high densities for establishment [27, 28]. Existing data for fruit flies are not conclusive regarding the best invasion strategy (damaged or undamaged fruit first), but the species' competitive abilities may indicate the potential of each to persist and prosper under intra- or interspecific competition and to compare the advantages and disadvantages of arriving and consuming unhealthy fruit first or piercing healthy fruit first [29].

Several studies are recognized as classical investigations analyzing and discussing results emerging from interspecific competition in different insect taxonomic groups, such as *Callosobruchus* beetles, *Drosophila* fruit flies, and *Tribolium* beetles. These studies have highlighted that the essential driver of competition is interspecific competition for resources [30]. Competition for food in insects can be triggered mainly when density-dependent mechanisms act on the population [31]. In fruit flies, these mechanisms are observed mainly in ephemeral food substrates, principally fruits, creating the conditions for intra- and interspecific competition [32].

Invader species generally exhibit biological attributes capable of facilitating the colonization process and establishment in new areas, principally the demographic parameters of fecundity and survival and the ability to disperse and adapt to the new conditions imposed by the new environments [33]. Different geographical regions may have seasonal changes capable of altering insects' behavior in different ways [34]. Local characteristics in the newly invaded environments such as abiotic environmental conditions, including the availability and heterogeneity of suitable habitats, and the implementation of plantations or orchards, resulting in interactions with other native and co-occurring alien species, can determine the success or failure of a species over time [35]. Thus, invader species must adapt to the new environmental conditions when arriving in new areas since humidity, rainfall, photoperiod, wind, and most importantly, temperature will undoubtedly significantly influence the new insects' distribution, abundance, and behavior [34, 36]. Besides abiotic effects, the biotic effects of the newly invaded area, such as the presence of native natural enemies that can prevent and control invasive species that arrive in low densities, can be challenging for the establishment [37]. Suitable habitat is fundamental for establishing exotic species, which often reach more habitats after invading the first one [38].

The effect of intra- and interspecific competition on the pest *D. suzukii* has been evaluated in different studies [39–41]. Intraspecific competition is expected in the field since females of *D. suzukii* oviposit more than one egg in the same substrate [9], and the competition has been shown to affect their pupation [39]. Although it is known as a primary pest, the wound left on the fruit by *D. suzukii* can attract a new range of pests, exposing the fruit fly to the possibility of interspecific competition [21]. Studies of interspecific competition between *D. suzukii* and different species have shown different impacts [41–43], which raises the possibility that the effect of interspecific interaction is dependent on the species that co-occur with *D. suzukii*. Studies of interspecific competition between drosophilids may show that the same species does not always have a competitive advantage. In fact, a study investigating the competitive interaction between *D. suzukii* and *Z. indianus* showed that to some extent, the performance of each species might depend on the substrate where they developed [43]. However, a structured study to analyze the competition between *D. suzukii* and *D. melanogaster* showed that one species might have a significantly greater competitive advantage [42]. In that study, the presence of *D. melanogaster* significantly reduced the emergence and egg-laying of *D. suzukii* [42].

Understanding that an insect's selection of an oviposition site is a crucial decision with downstream consequences for population dynamics [44], this study evaluated the abundance of drosophilids co-occurring in the field with *D. suzukii* in Brazil. The study also assessed the effects of different intra- and interspecific densities, as a proxy for competition, on the oviposition behavior of *D. suzukii* females and the interactions between the coexisting competitors *D. suzukii* and *Z. indianus*.

2.2 Material and Methods

2.2.1 Fruit sample collections and drosophilid diversity assessment

Strawberry fruits (*Fragaria* x *ananassa*), cultivar 'Festival' (GCREC-Dover, 1995) [45] were collected from a strawberry field with seedlings transplanted on March 29, 2020, in Atibaia municipality, São Paulo (23°04'16"S, 46°40'52"W). Strawberry plants were cultivated in open beds 40 m long, in a total area of 0.2 ha. Plants were drip-irrigated every two days. The fruits were collected twice: in October (130 fruits) and December (150 fruits) of 2020. Overripe fruits were collected from the ground and brought to the laboratory to assess the emergence of drosophilids. The fruit was collected randomly around the strawberry field, at 13 places for the first collection and 15 places in the second collection, with ten fruits collected at each place. Overripe fruits were chosen for field collection because the study's primary goal was to detect the presence of both species co-occurring in the field. The results indicated that both species can occur simultaneously in the same fruit [46]. The fruits were maintained in the laboratory under controlled conditions (R.H. = 60%, L:D = 12:12, T = 26°C). They were placed in 500-mL plastic pots with a layer of vermiculite to absorb moisture, with ten fruits in each pot. For 16 days, the emergence of drosophilids was evaluated daily, and emerging insects were identified under a stereoscopic microscope, using a dichotomous key [47] and the taxonomic description by Van der Linde [48]..

2.2.2 *Drosophila suzukii* and *Zaprionus indianus* intraspecific and interspecific competition bioassays

2.2.2.1 Insect rearing:

Individuals of *D. suzukii* were obtained from a laboratory colony of Embrapa Clima Temperado, Pelotas, São Paulo (31°48'13.96"S 52°24'41.40"W). Individuals of *Z. indianus* were obtained from guava fruits collected in the fields at the Escola Superior de Agricultura (ESALQ; 22°42'30"S 47°38'30"W). The insects used were kept in the artificial diet for at least three generations prior to the bioassay, under controlled conditions (R.H. = 60%, L:D = 12:12, T = 26°C). The artificial diet used for insect rearing and the bioassay followed the artificial diet suggested by Andreazza (2016) [49], composed of cornmeal, sugar, brewer's yeast, agar, propionic acid, and Nipagin®. All the laboratory colonies were kept and the bioassays were conducted in the laboratory in controlled conditions (R.H. = 60%, L:D = 12:12, T = 26°C). The diet was previously tested in populations of *Z. indianus* to assure the same survival conditions as *D. suzukii*, preventing factors other than competition from influencing the survival of *Z. indianus*.

2.2.2.2 Development bioassay:

The effect of different egg densities on the developmental parameters of *D. suzukii* and *Z. indianus* was assessed under inter- and intraspecific competition. Eight densities (ranging from 8 to 400), each with 5 repetitions, were tested using the artificial diet [49]. Plastic cups with a volume of 50 mL were filled with 15 g of artificial diet. After 24 h the diets were inoculated by manually transferring eggs of *D. suzukii* and *Z. indianus* for the interspecific competition, or eggs of only *D. suzukii* or only *Z. indianus* for the intraspecific competition, to form the densities: 0.55, 0.69, 1.38, 2.76, 4.14, 6.89, 13.79, or 27.59 eggs/g of diet (i.e., 8, 10, 20, 40, 60, 100, 200, or 400 eggs per cup). The percentages of egg-pupa and pupa-adult viability were measured by counting the pupae and adults, respectively. The mean development time was calculated as the number of days from egg to adult, and the sex ratio was assessed based on counts of all adults 48 h after emergence.

2.2.2.3 Fecundity bioassay:

To assess the effects of inter- and intraspecific competition on female fecundity, all adults that emerged from the development experiment were separated into larger plastic cages (380 mL), with one cage for each repetition at each density and allowed them to reach maturity for twenty-four hours for *D. suzukii* and four days for *Z. indianus*. These intervals were based on our laboratory observations in preliminary tests with the different densities where the female flies showed oviposition-probing behavior as reported elsewhere [9, 50, 51]. In the case of *D. suzukii*, such behavior was considered as an initial potential gateway for its competitor *Z. indianus*. After this maturation period, a 50-mL plastic cup with 15 g of the artificial diet free of eggs was added to the cage, and adults were allowed to oviposit on the diet for 24 h. Then the diet cup was replaced with a new one and the oviposited eggs were counted under a stereoscopic microscope. This process was repeated for six consecutive days, a period during which we observed a decrease in the eggs laying by the females of *D. suzukii*, the primary pest in the substrate (S1 Table). Fecundity was estimated based on the number of eggs per female in each treatment.

2.2.2.4 Leslie matrix

The Leslie matrix was used to describe the population growth, taking into account the stages [52] of *D. suzukii* and *Z. indianus*. The biological parameters in the matrix were survival and fecundity, which describe changes in population size based on the changes in their values [53, 54]. The model can be represented by the equation:

$$X_{t+1}=Ax_t$$

where *X* determines the population size at time *t+1* as a function of time *t* and *A* represents the n × n matrix:

$$A = \begin{bmatrix} F1 & F2 & F3 & F4 \\ S1 & 0 & 0 & 0 \\ 0 & S2 & 0 & 0 \\ 0 & 0 & S3 & 0 \end{bmatrix}$$

The first line in the matrix indicates "F" values, determining the fecundity of the individuals in each age stage, and the diagonal with "S" values indicates the survival of individuals between life stages [52].

In the equation, x_t represents the stage of the individual present at time *t*, with population growth. The Leslie matrix A is the equation term governing the population growth. The initial values for each age or stage are given by the column matrix, written as:

$$x_{t=} \begin{bmatrix} a \\ b \\ c \end{bmatrix}$$

Each row of the column matrix represents the initial density of an insect life stage. In the present study, only the first row was filled, with the initial egg density.

Based on the evaluation of data from the development and fecundity bioassay employing the Leslie Matrix with 10 time-step projections, three densities were selected of the eight densities tested, to represent a low (8 eggs), a medium (60 eggs), and a high (400 eggs) density scenario to define the matrix model conditions.

2.2.3 Behavior bioassay

2.2.3.1 Choice behavior of *Drosophila suzukii* with eggs of *Zaprionus indianus* or with eggs of *D. suzukii*:

A free-choice assay was used to evaluate the effects of the presence of inter- or intraspecies eggs on the oviposition behavior of *D. suzukii* females. In 100-mL plastic cages, combinations of egg-infested diet at different densities versus a non-infested diet (control) were tested. A mean of 0.53 g of diet placed in Eppendorf caps was manually infested with eggs from diets of the laboratory colony of *Z. indianus* or *D. suzukii*. Four egg densities (1, 3, 7, or 15 eggs per diet, i.e., 1.88, 5.66, 13.20, or 28.30 eggs per gram) were used, each with 9 repetitions. Next, two diets were placed in a cage, one free of eggs and the other with eggs of one of the species at the density tested. Five females and three males of *D. suzukii* three days old were released into the cage and left in contact with the diets for 24 h under laboratory conditions (R.H. = 60 ±5%, L:D = 12:12 h, T = 26±1°C). After this period the diets were removed and the eggs counted under a stereoscopic microscope. Because of the morphological difference between the eggs of each pest, i.e., *D. suzukii* eggs have two respiratory filaments while *Z. indianus* eggs have four respiratory filaments [55], it was possible to evaluate the interspecific density. The initial density, manually infected for the bioassay, was subtracted from the final count of eggs in the oviposition behavior assays.

2.2.4 Statistical analysis and Leslie matrix approach

The data for development and fecundity for intra- and interspecific competition and the data for mean development time were submitted to regression with the curve adjusted to best fit, using the curve-fitting procedure of SigmaPlot v. 12.5. Linear, quadratic, inverse first-order polynomial curves, and exponential growth and decay models were tested to determine the level of significance and R^2 values. Model selection was based on parsimony (i.e., simplest model with highest adjusted R^2 value), high F-values, and steep (relative) increases in R^2 with model complexity. The data for fecundity and development of 3 densities (8, 60, and 400 eggs) were also submitted to a Leslie matrix with a projection of 10 time steps. The data from the two-choice bioassay of intra- and interspecific behavior were analyzed by Student's *t*-test. The Leslie matrix analysis was performed using R software, and the other analyses were performed using SigmaPlot v. 12.5 (Systat Software, San José, CA, USA).

2.3 Results

2.3.1 Field collections

Zaprionus indianus generally outnumbered the other species. For the first collection date, the total of 836 adults included 12 *D. suzukii* (1.43%), 692 *Z. indianus* (82.77%), and 132 other *Drosophila* species (15.78%). In the second collection, 948 adults emerged, including 26 *D. suzukii* (2.74%), 790 *Z. indianus* (83.33%), and 132 other *Drosophila* species (13.92%) (Fig 1).

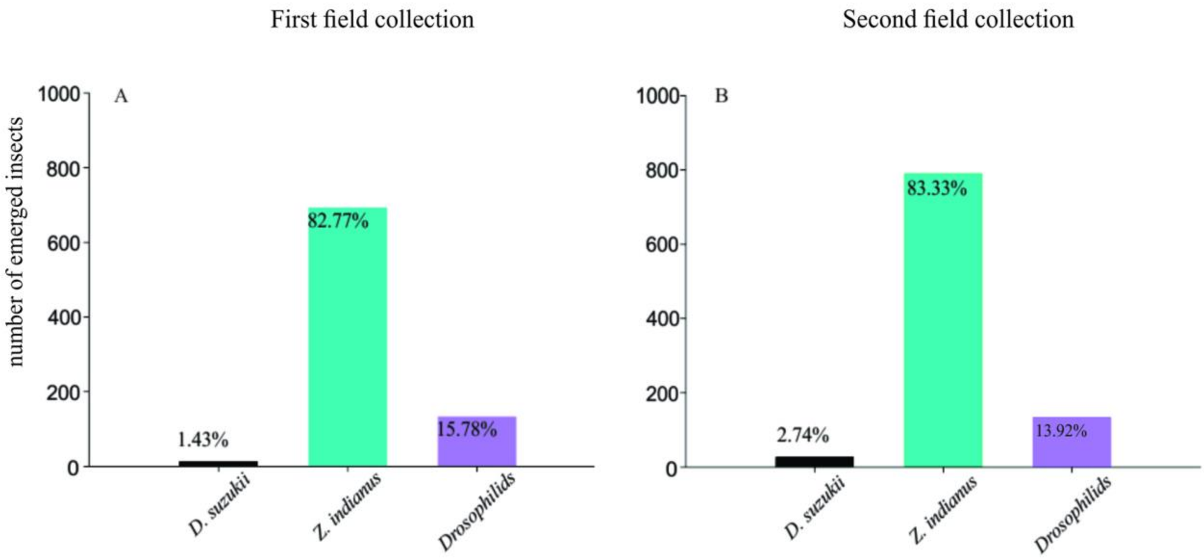


Fig 1. Fruit sample collections and drosophilid diversity assessment.
Number of insects emerged from strawberry fruits collected from the field in Atibaia municipality, São Paulo, Brazil (23°04'16"S, 46°40'52"W) in October (A) and December (B) 2020.

2.3.2 Intraspecific and interspecific competition

2.3.2.1 Development bioassay:

A linear-regression analysis showed that the effect of density depended on the competing species as well as on the type of competition, intra- or interspecific (Fig 2).

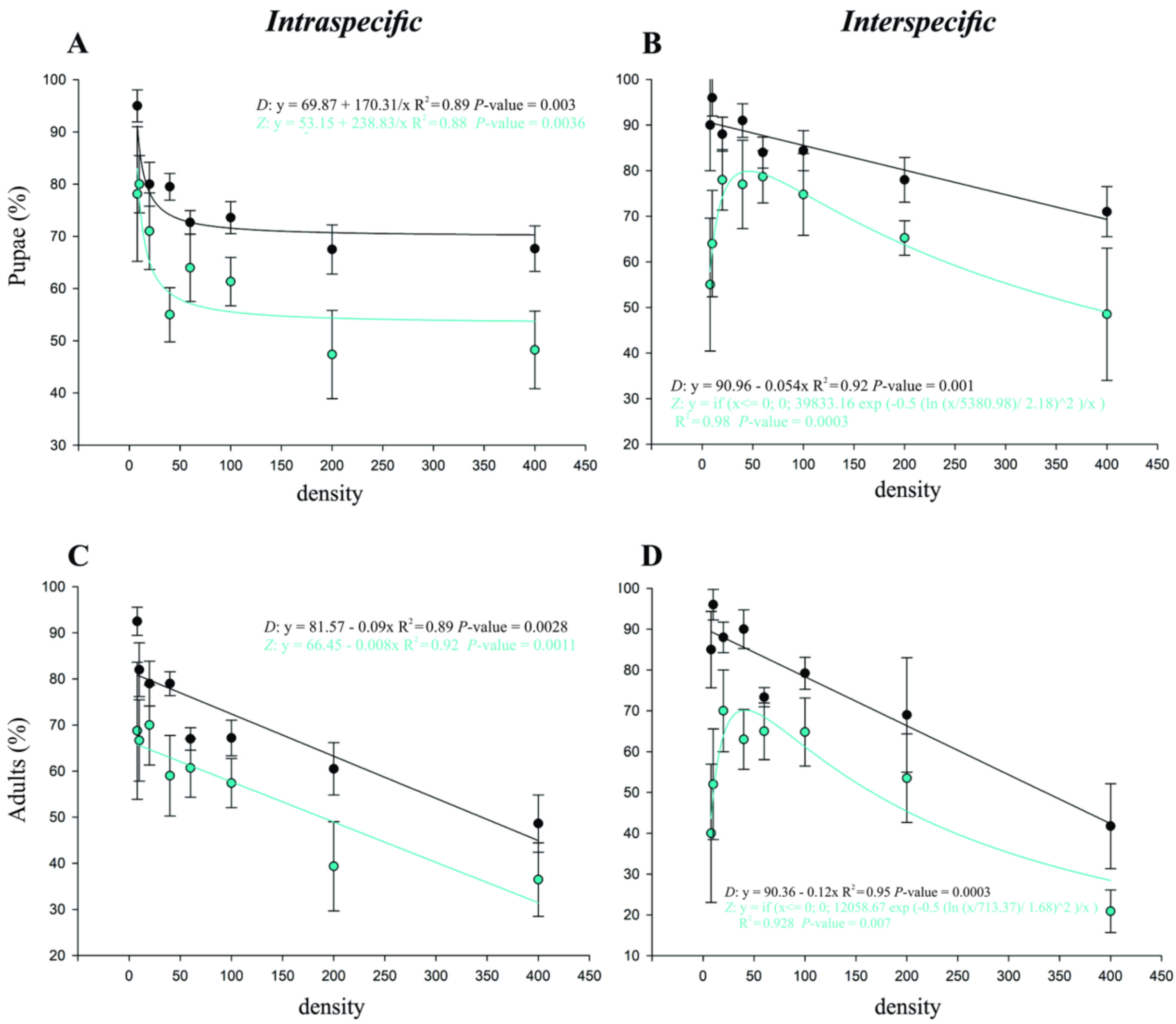


Fig 2. Development bioassay.

Percentages of egg-pupa and pupa-adult viability of *Drosophila suzukii* (D) and *Zaprionus indianus* (Z) fitted to regression functions at eight intra- and interspecific egg densities, ranging from 8 to 400. (A) Egg-pupa intraspecific survival. (B) Egg-pupa interspecific survival. (C) Intraspecific emergence percentage of adults. (D) Interspecific emergence of adults.

Under intraspecific competition, a density-dependent decrease in egg-pupa survival was observed for *D. suzukii* and *Z. indianus* (Fig 2A). The data for both species, adjusted to the inverse first-order polynomial curve, showed that an increase in egg density resulted in an initial reduction of pupa survival at lower densities, followed by stabilization of pupa survival at higher densities (Fig 2A, Table 1).

	Density	Model	Treatment	Estimated parameters			df_{error}	F	p	R^2
				a	b	y_0 or x_0				
% of pupae	*intraspecific*	$Y = y_0+(a/x)$	*D. suzukii*	170.31(83.29–257.32)	-	69.87 (64.62–75.12)		22.93	0.003	0.89
		$Y = y_0+(a/x)$	*Z. indianus*	238.63(112.25–365.4)	-	53.15 (45.51–60.80)		21.31	0.004	0.88
	interspecific	$Y = y_0+a^*x$	*D. suzukii*	−0.05(−0.07– −0.03)	-	90.96 (87.34–94.58)	7	36.06	0.001	0.92
		$Y = a^*exp(-0.5^*(ln(x/x_0)/b)^2)/x)$	*Z. indianus*	39833.1(17402.6–62263.6)	2.18(1.91–2.45)	5380.9 (−917.07–11679.03)	7	62.68	0.0003	0.98
% emergence	*intraspecific*	$Y = y_0+a^*x$	*D. suzukii*	−0.09(−0.13– −0.04)	-	81.57(74.03–89.11)	7	23.85	0.003	0.89
		$Y = y_0+a^*x$	*Z. indianus*	−0.08(−0.12– −0.05)	-	66.45(60.44–72.45)	7	34.24	0.001	0.92
	interspecific	$Y = y_0+a^*x$	*D. suzukii*	−0.12(−0.15– −0.08)	-	90.36 (84.03–96.68)	7	58.09	0.0003	0.95
		$Y = a^*exp(-0.5^*(ln(x/x_0)/b)^2)/x)$	*Z. indianus*	12058.6(3044.9–21072.3)	1.68(1.21–2.15)	713.37 (−418.1–1844.9)	7	15.7	0.007	0.92

https://doi.org/10.1371/journal.pone.0281806.t001

Table 1. Summary of regression analyses for percentages of egg-pupa and pupa-adult viability and adult emergence of *Drosophila suzukii* and *Zaprionus indianus* (shown in Fig 2).

For both species, the percentage of adult emergence showed a decreasing density-dependent pattern, with a noticeable change at high densities (Fig 2C). Adult emergence followed a polynomial linear trend (Fig 2C, Table 1), decreasing continuously even at densities of 13.79 and 27.59 eggs/g of diet (200 and 400 eggs per diet).

With interspecific competition, the patterns of egg-pupa survival and adult emergence changed (Fig 2B and 2D). While *D. suzukii* showed a linear density-dependent decrease in both egg-pupa survival and adult emergence, *Z. indianus* showed a bell-shaped curve with a peak between density 40 and density 60, indicating that this density range is optimal for the survival of the egg through pupa stages and adult emergence for this species (Fig 2B and 2D, Table 1).

For both cases, intraspecific and interspecific competition, and for both development phases, egg-pupa and adult emergence, the curve for *D. suzukii* was higher than the curve for *Z. indianus*, i.e., with higher values.

2.3.2.2 Fecundity bioassay:

In the intraspecific competition, the data fitted to the inverse first-order polynomial model indicated that the density increase leads to a decrease in female fecundity in each species (Fig 3A, Table 2). However, in the interspecific competition and independently of the species, the fit of fecundity data to the peak-type curves indicated a maximum fecundity per female of approximately 40 eggs for *Z. indianus* and 10 eggs for *D. suzukii*. Notably, the fecundity per female of *Z. indianus* was higher than that of *D. suzukii* for all densities tested (Fig 3B, Table 2).

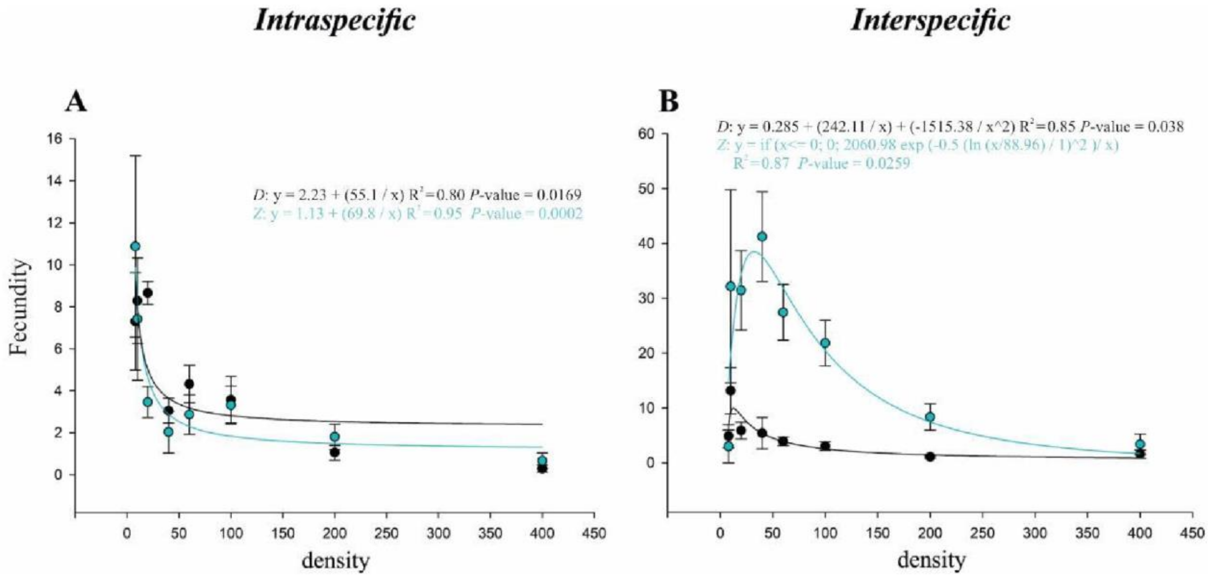


Fig 3. Fecundity bioassay.

Fecundity response of *Drosophila suzukii* (D) and *Zaprionus indianus* (Z) fitted to regression functions to eight intra- and interspecific egg densities, i.e., 8 to 400 eggs. (A) Intraspecific mean fecundity per female. (B) Interspecific mean fecundity per female. Black points and curves represent *D. suzukii*; green points and curves represent *Z. indianus*.

Variable	Model	Treatment	Estimated parameters			df_{error}	F	p	R^2
			a	b	y_0 or x_0				
Intraspecific fecundity	$Y = y_0+(a/x)$	*D. suzukii*	55.1 (13.97–96.22)	-	2.23 (−0.21–4.74)	7	10.74	0.016	0.80
	$Y = y_0+(a/x)$	*Z. indianus*	69.8 (48.9–90.7)	-	1.13 (−0.12–2.4)	7	66.85	0.0002	0.95
Interspecific fecundity	$Y = y_0+(a/x) + (b/x^2)$	*D. suzukii*	242.1 (21.25–462.98)	−1515.3 (−3256.5–225.74)	0.28 (−3.65–4.22)	7	6.71	0.0383	0.85
	$Y = a^*\exp(-0.5^*(\ln(x/x_0)/b)^2)/x)$	*Z. indianus*	2060.9 (828.63–3293.3)	1 (0.51–1.5)	88.96 (−11.62–189.55)	7	8.27	0.02	0.87

https://doi.org/10.1371/journal.pone.0281806.t002

Table 2. Summary of regression analyses for fecundity of *Drosophila suzukii* and *Zaprionus indianus* (shown in Fig 3).

2.3.2.3 Mean development time:

The intraspecific and interspecific competition trajectories for *D. suzukii* were significantly fitted to a linear regression (Table 3). The development time increased with the increase in density, with a mean difference of 4 days between the highest and lowest densities. With respect to the type of interaction, intra- or interspecific, even though the intraspecific trajectory was higher than the interspecific line, the development times overlapped (Fig 4A, Table 3). Similarly to *D. suzukii*, the egg-adult development time for *Z. indianus* increased with the increase in density, with a mean difference of 4 days and 6.88 days from the lowest and the highest density tested for intra- and interspecific competition, respectively (Table 3). The mean development time of *Z. indianus* showed a regressive inversion pattern compared to the regressions of *D. suzukii*, where now the mean development time of *Z. indianus* was longer under interspecific competition than under intraspecific competition (Fig 4B, Table 3).

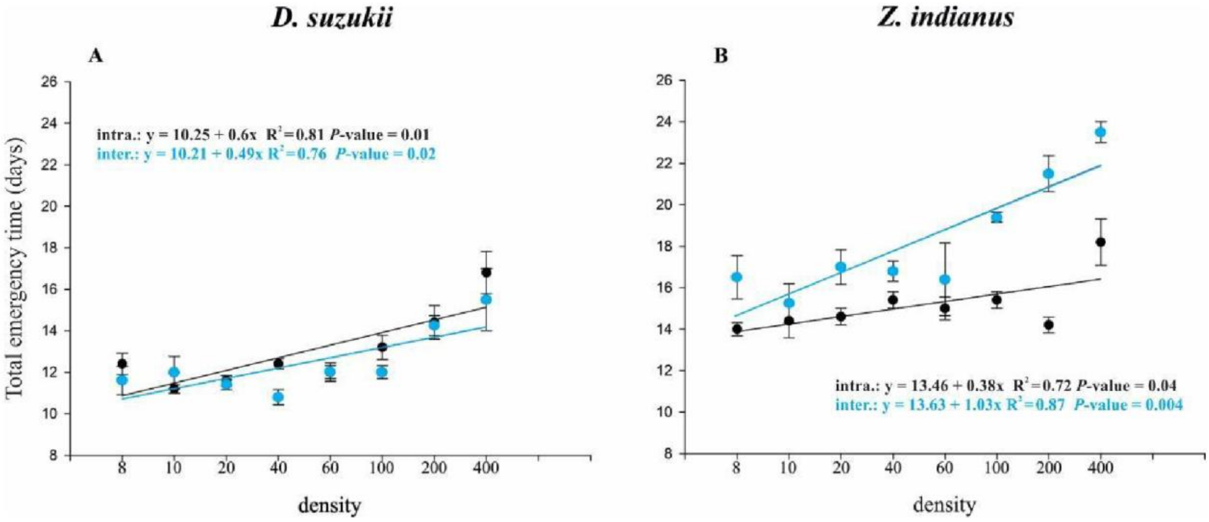


Fig 4. Development time.

Mean emergence times of *Drosophila suzukii* (D) and *Zaprionus indianus* (Z) fitted to regression functions in the 8 different densities. The black line and points represent intraspecific competition; the blue line and points represent interspecific competition.

Variable	Model	Treatment	Estimated parameters		df_{error}	F	p	R^2
			a	y_0 or x_0				
D. suzukii	$Y = y_0+a*x$	*intraspecific*	0.6 (0.18–1.03)	10.25 (8.08–12.42)	7	12.07	0.01	0.81
	$Y = y_0+a*x$	*interspecific*	0.49 (0.07–0.91)	10.21(8.09–12.33)	7	8.3	0.02	0.76
Z. indianus	$Y = y_0+a*x$	*intraspecific*	0.38 (0.01–0.75)	13.46 (11.61–15.21)	7	6.61	0.04	0.72
	$Y = y_0+a*x$	*interspecific*	1.03 (0.47–1.60)	13.63 (10.77–16.48)	7	20.08	0.004	0.87

https://doi.org/10.1371/journal.pone.0281806.t003

Table 3. Summary of regression analyses for mean emergence times of *Drosophila suzukii* and *Zaprionus indianus* (shown in Fig 4).

2.3.3 Leslie matrix:

For *D. suzukii* under intraspecific competition, the characteristics of the trajectory indicated a growth trend of the population as a projection over time for the low-density scenario (Fig 5A). The same growth pattern was apparent in the trajectory under medium density, but with a reduction to half of the final population size. Under high density, the population tended to decrease with time (Fig 5A). A similar pattern was apparent in interspecific competition, with a reduction of the population size compared with the intraspecific competition (Fig 5A).

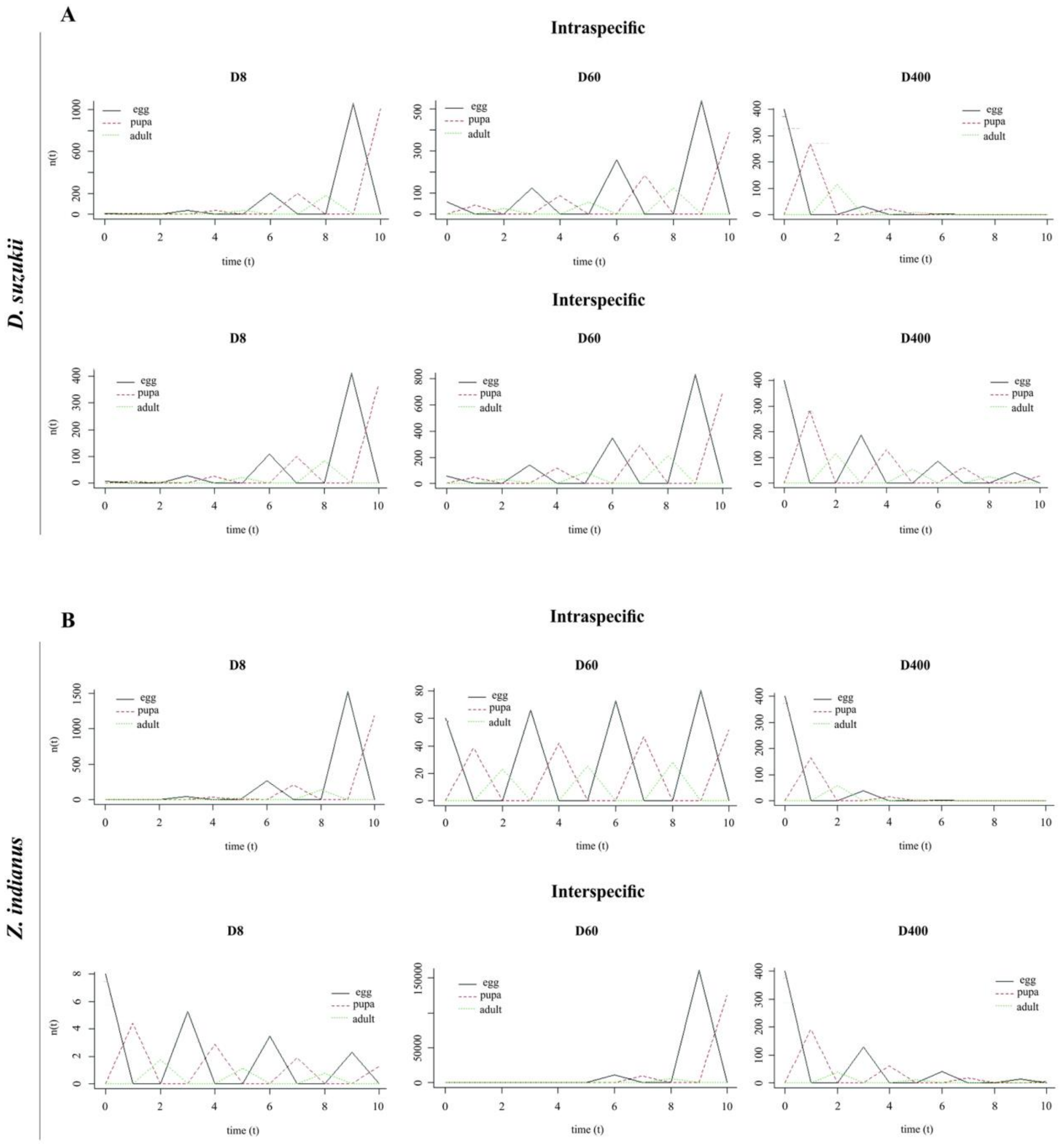


Fig 5. Leslie matrix simulations showing population sizes for life stages of *Drosophila suzukii* (A) and *Zaprionus indianus* (B) from intra and interspecific combinations in scenarios of low (D8), moderate (D60) and high (D400) densities.

For *Z. indianus* under intraspecific competition and in the low-density situation, the pattern of the trajectory was similar to *D. suzukii* (Fig 5B). Under medium density, cyclic oscillations of population growth were seen for all stages of *Z. indianus* (Fig 5B). Under high density, as was observed for *D. suzukii*, the population growth declined over time. In the interspecific competition, a different pattern was apparent for low density, where, as for high density, the population growth declined (Fig 5B).

2.3.4 Behavior bioassay:

In the two-choice bioassay, independently of the density, no significant differences were found in the number of eggs laid by *D. suzukii* females allowed to oviposit on either egg-infested or control artificial diet (*P*-value > 0.05, Fig 6A). Similarly, *D. suzukii* showed no preference between the control diet and the diet that was previously infested with eggs of *Z. indianus* at any of the densities tested (*P*-value > 0.05, Fig 6B). The mean number of eggs laid was between 6.33 ±1.54 (density 1) and 8.77 ±1.54 (density 10) for the control diet, and between 5.11 ±1.35 (density 1) and 11.11 ± 2.53 (density 3) for diets that were previously infested with *D. suzukii* eggs.

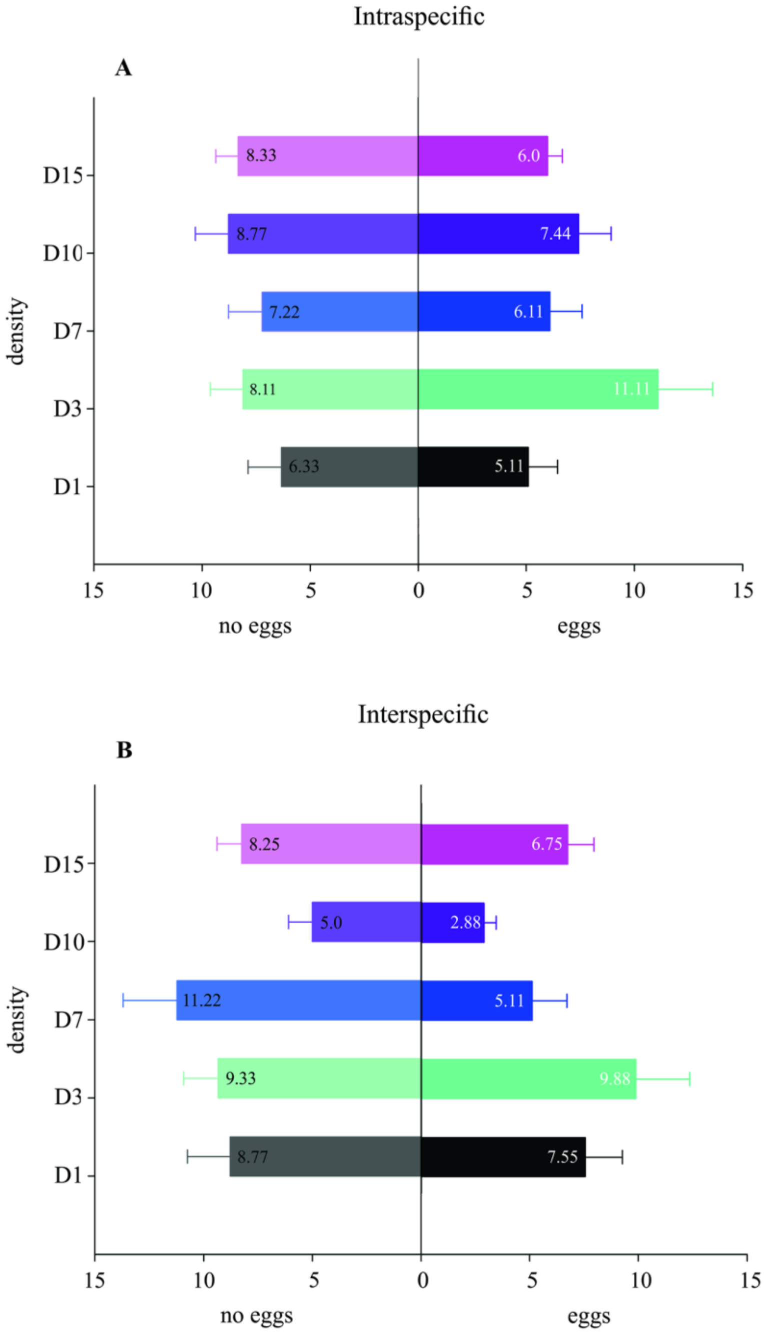


Fig 6. Choice behavior of *Drosophila suzukii* with eggs of *Zaprionus indianus* or with eggs of *D. suzukii*.

Experiments indicating the total eggs laid by *D. suzukii* on a diet previously infested with different egg densities (1, 3, 7, 10, or 15 eggs) versus a blank diet. (A) Intraspecific: oviposition behavior of *D. suzukii* in one diet with eggs of *D. suzukii* versus a blank diet (no eggs). (B) Interspecific: oviposition behavior of *D. suzukii* in one diet with eggs of *Zaprionus indianus* versus a blank diet (no eggs).

Similarly, to the intraspecific bioassay, in the bioassay with eggs of *Z. indianus*, independently of the density, *D. suzukii* showed no preference (*P*-value > 0.05, Fig 6B). The mean number of eggs laid was between 5.0 ±1.09 (density 10) and 11.22 ±2.48 (density 7) for the control diet, and between 2.88 ±0.56 (density 10) and 9.88 ±2.47 (density 3) for diets previously infested with *Z. indianus* eggs.

2.4 Discussion

The two drosophilid species *D. suzukii* and *Z. indianus* occur together in several regions of the world, including Brazil [55–59]. When co-occurring, *Z. indianus* is consistently found to significantly outnumber *D. suzukii* [43]. Our field collections of strawberry fruits provided substantial evidence concerning the co-occurrence of the two species previously reported, with a higher number of *Z. indianus* than *D. suzukii* [60, 61]. Nevertheless, a study focusing on field collections with fruits and attractant-baited traps should be encouraged to assess the differences in both species' population sizes. Under these density conditions, both interspecific and intraspecific competitions are expected to induce changes in important fitness traits of these species. Based on these numerical differences between the two species and knowing that *Z. indianus* is an opportunist that requires naturally damaged or previously infested fruits to complete its life cycle [1], we experimentally investigated the roles of competition types (inter- and intra-) in the survival and development dynamics of the two species. Our results from laboratory experiments showed that the biology of *D. suzukii* was affected by egg density and partially by the competition type (intra- and interspecific), mainly when observing an inversion of fecundity values, indicated by a higher fecundity of *Z. indianus* in interspecific densities. However, the oviposition behavior of *D. suzukii* females was not affected by the previous presence of *Z. indianus* eggs or by the egg density in the oviposition substrate. On the other hand, *Z. indianus* showed higher fecundity in the presence of *D. suzukii* eggs. This result suggests that interspecific competition could be more advantageous for *Z. indianus* than intraspecific competition.

Our findings showed that independently of the competition type and species, increasing densities had an overall negative effect on development time and fecundity. The emergence time of *Z. indianus* was clearly influenced by the type of competition, increasing in interspecific cultures; in contrast, *D. suzukii* emerged after similar time periods in both intra- and interspecific cultures. The population density of conspecifics is known to affect individual oviposition rates, impacting fecundity and offspring fitness under resource scarcity [62]. Takahashi and Kimura [63] evaluated the effect of intraspecific competition on the per-capita egg production of *D. suzukii* (fecundity). They found a decrease in egg production per capita with the increase in density. Our study also showed a decrease in fecundity with an increase in density. However, we found a similar reduction of *D. suzukii* fecundity whether alone or in competition with *Z. indianus*.

The effect of interspecific competition on *D. suzukii* fitness has been studied mostly with the common fruit fly *Drosophila melanogaster* Meigen as a competitor. In direct interspecific-competition situations, previous oviposition by *D. melanogaster* was shown to deter female *D. suzukii* from ovipositing in the same substrate [41, 42]. Furthermore, under these interspecific conditions, *D. suzukii* larvae showed lower survival than *D. melanogaster* larvae [64]. In assays of interspecific competition between *D. suzukii* and *Z. indianus*, using both an artificial diet and different grape cultivars, the outcome of the competition depended on the oviposition and development substrate [43]. High densities of *Z. indianus* did not affect the mortality and development of *D. suzukii* when an artificial diet was used, as opposed to grapes, where higher mortality rates were observed [43]. We suppose that the effect of the oviposition substrate would be more marked in the field, since the experiment was conducted with an artificial diet containing a standard quantity of nutrients, differently from field conditions with a natural fruit-based diet [40, 43].

Zaprionus indianus responded to the interspecific-density effect with a hump-shaped relationship around densities corresponding to 1.33 and 2.66 eggs/g of diet, indicating a positive density-dependence effect. In this study, the artificial diet was employed to avoid the influence of unknown factors associated with any imbalances in the chemical content of fruit capable of influencing the competitive performance. With the standardized diet, we believe that we could more precisely analyze the effect of competition on the development, fecundity, and behavior of the pests. When showing a competition effect occurring with as many controlled conditions as possible, we presume that the effect found would be accentuated in field conditions.

It is also noteworthy highlighting that in our fecundity experiment; we used individuals, less than one day old, resulting from the previous experiment with different densities (ranging from 8 to 400) that were observed during a 6 days oviposition period. Such experimental conditions could explain some divergences from previous literature [65–68] regarding the oviposition behavior of the studied drosophilids. In fact, biotic and abiotic experimental conditions have been reported to differentially affect the development, behavior, phenology, and reproductive biology of *D. suzukii* [69].

In the present study, the results from the projections of the Leslie matrix, although produced through computer simulations, provide evidence for a hypothesis capable of explaining the ecological patterns of the oscillations observed. Allee or hydra effects could be interesting issues for investigation, to explain how low abundance, or as in this study, density dependence causing mortality, would subsequently benefit a population by increasing its equilibrium, especially in cycling populations [70, 71]. Here, the population oscillation patterns observed from the projections were practically the same when analyzing simulations of intra- and interspecific population dynamics for *D. suzukii*. That is, increasing oscillations at the densities of 8 and 60 and decreasing oscillations at the density of 400 were observed. However, the oscillation pattern of *Z. indianus* in response to intra- and interspecific competition differed significantly from the pattern of *D. suzukii*, except at the density of 400. At the intraspecific density of 8, the oscillation pattern was similar to *D. suzukii*, but in interspecific simulations, the *Z. indianus* population showed a decreasing and oscillating trend. At the density of 60, the intraspecific oscillation showed a slight, gradual increase in the range of oscillation, but under interspecific competition conditions, the oscillation pattern was reversed.

Another critical aspect of these results is the population peaks of these flies in intra- and interspecific simulations. In intraspecific densities of *D. suzukii*, the peak numbers of eggs, pupae, and adults decreased with increasing densities, showing a density-dependence effect for all life stages. However, in interspecific densities of *D. suzukii*, the peak numbers of eggs, pupae, and adults increased from a density of 8 to 60, and decreased only at the density of 400. The peaks of *Z. indianus* decreased dramatically in intraspecific simulations from 8 to 60, and slightly from 60 to 400. However, in interspecific densities, the *Z. indianus* peaks increased strongly from 8 to 60, with a significant decrease from 60 to 400. Therefore, intraspecific densities clearly exert stronger negative density-dependent effects than interspecific densities for both species. This result suggests than the co-occurrence between *D. suzukii* and *Z. indianus* could be beneficial for the persistence of both species. To the best of our knowledge, no studies have combined competitive interactions with the Leslie matrix, as performed here. The Leslie matrix has traditionally been employed to elucidate other aspects of demography, population dynamics, and theoretical ecology, using flour beetles, blowflies, and stinkbugs [30, 72, 73]. Applications of the Leslie matrix to *D. suzukii*, *Z. indianus*, or other drosophilids have investigated only questions associated with life tables; development, emphasizing temperature; or environmental conditions.

In the two-choice bioassays, the egg density did not appear to affect the oviposition behavior of *D. suzukii*, as females showed no preference for diets infested or not with conspecific eggs or *Z. indianus* eggs, diverging from a previous report that *D. suzukii* females avoided ovipositing in a substrate pre-inoculated with eggs of *D. melanogaster* [42]. This difference in behavior could be explained by the ability of some species to mark the oviposition substrate with pheromones. Indeed, males of *D. melanogaster* produce the pheromone cis-vaccenyl acetate (cVA) as a marker of the oviposition substrate, which plays a role in different behavioral activities of *Drosophila*, such as male-male aggregation, a reproductive-isolation mechanism; and in modulating oviposition behavior [74, 75]. Although *D. suzukii*, *Z. indianus*, *D. rufa*, and *D. auraria* (among others) do not produce this compound [75], they may still be able to recognize it [76], explaining *D. suzukii*'s avoidance of substrates previously infected with *D. melanogaster* eggs and the lack of this effect with *Z. indianus* eggs.

Density dependence has been considered a significant factor influencing biological invasion processes [77]. A high competitive ability has been mentioned as the principal factor responsible for the success of invader species because it allows high rates of population growth [19]. Commonly, competition has been studied by confronting invaders with local species, with results that frequently indicate that the invaders are superior competitors [78, 79]. The present study analyzed two invading species, and therefore both species might maintain the status of the superior competitor. The only difference in terms of advantage would be the time since the invasion event, assuming that the earlier-arriving species would have more time to adjust to its new habitat [80]. Our experimental results provide evidence that competition between *D. suzukii* and *Z. indianus* limits the numbers of *D. suzukii*, suggesting that even between two invader species, larval competition can result in a significant difference in their competitive ability.

In addition to competitive abilities at different intra- and interspecific densities, other factors not investigated in this study can also influence the abundance of drosophilids. Survivorship in drosophilids is associated with temperature. *Zaprionus indianus* withstands high temperatures in regions with wide climate variation, such as Rio Grande do Sul, Brazil [81], although *D. suzukii* also shows a degree of tolerance to a tropical climate [82]. Physicochemical characteristics of fruits also seem to affect the establishment and co-occurrence or repellence of both species [50].

In conclusion, our study demonstrated that for *D. suzukii*, the presence of *Z. indianus* can be considered neutral, since the oviposition behavior of the former did not change in the presence of different densities of *Z. indianus*, remaining similar to the behavior in the presence of its own species. The survival and fecundity of *D. suzukii* maintained the same pattern in the presence of conspecifics or *Z. indianus*, also indicating the lack of effect of *Z. indianus* on *D. suzukii*. On the other hand, *Z. indianus* seems to have produced more offspring in the presence of *D. suzukii* than with its conspecifics. These results may explain the high densities of *Z. indianus* in the field together with *D. suzukii* species. This study highlights the importance of understanding the consequences of the interactions in the field, to anticipate whether these interactions may be transient or permanent. A better understanding of these interactions can help to develop more-appropriate management techniques, anticipating what may occur in the field and thus controlling pests more efficiently.

This study showed how co-occurring fruit flies can use different strategies involving responses in life-history parameters, such as survival and fecundity, when under intra- or interspecific conditions. The responses to these conditions may help to explain the different patterns of coexistence on local and global scales, emphasizing differences among regions and host fruits and providing insights for planning for new crops, especially orchards. Significant evidence supports the idea that competitive interactions can be intensified with climate change, particularly in tropical areas, and new ecological patterns of coexistence and co-occurrence can emerge from this new scenario [83, 84], certainly impacting the distribution of flies in South America and around the world.

References

1.van der Linde K, Steck GJ, Hibbard K, Birdsley JS, Alonso LM, Houle D. First records of *Zaprionus indianus* (Diptera: Drosophilidae), a pest species on commercial fruits from Panama and the United States of America. Fla Entomol. 2006;89:402–404.

2.Lavagnino NJ, Fanara JJ, Mensch J. Comparison of overwintering survival and fertility of *Zaprionus indianus* (Diptera: Drosophilidae) flies from native and invaded ranges. J Therm Biol. 2020;87:102470. pmid:31999602

3.Vilela CR. Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? Drosoph Inf Serv. 1999;82:37–39.

4.Kato CM, Foureaux LV, César RA, Torres MP. Ocorrência de *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) no estado de Minas Gerais. Ciência e Agrotecnologia. 2004;28:454–455.

5.Leão BFD, Tldon R. Newly invading species exploiting native host-plants: The case of the African *Zaprionus indianus* (Gupta) in the Brazilian Cerrado (Diptera, Drosophilidae). Ann Soc Entomol Fr. 2004;40:285–290.

6.Santos JF, Rieger TT, Campos SRC, Nascimento ACC, Félix PT, Silva SVO, et al. Colonization of northeast region of Brazil by the drosophilid flies *Drosophila malerkotliana* and *Zaprionus indianus*, a new potential pest for Brazilian fruit culture. Drosoph Inf Serv. 2003;86:92–95.

7.Deprá M, Poppe JL, Schmitz HJ, De Toni DC, Valente VLS. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J Pest Sci. 2014;87:379–383.

8.Asplen MK, Anfora G, Biondi A, Choi D. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci. 2015;469–494.

9.Kanzawa T. Studies on *Drosophila suzukii* Mats. Stud Drosoph suzukii Mats. 1939.

10.Lee JC, Bruck DJ, Dreves AJ, Loriatti C, Vogt H, Baufeld P. In focus: spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Manag Sci. 2011;67:1349–1351.

11.Calabria G, Máca J, Bächli G, Serra L, Pascual M. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J Appl Entomol. 2012;136:139–147.

12.Kirschbaum DS, Funes CF, Buonocore-Biancheri MJ, Suárez L, Ovruski SM. The biology and ecology of *Drosophila suzukii* (Diptera: Drosophilidae). In: Garcia FRM, editor. *Drosophila suzukii* Management. Cham: Springer; 2020. pp. 41–91.

13.Bragard C, Baptista P, Chatzivassiliou E, Di Serio F, Gonthier P, Jaques Miret JA, et al. Pest categorisation of *Zaprionus indianus*. EFSA J. 2022;20. pmid:35281655

14.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. Bull Insectology. 2012;65:149–160.

15.Tochen S, Dalton DT, Wiman N, Hamm C, Shearer PW, Walton VM. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ Entomol. 2014;43:501–510. pmid:24612968

16.Grumiaux C, Andersen MK, Colinet H, Overgaard J. Fluctuating thermal regime preserves physiological homeostasis and reproductive capacity in *Drosophila suzukii*. J Insect Physiol. 2019;113:33–41. pmid:30615858

17.Wong JS, Cave AC, Lightle DM, Mahaffee WF, Naranjo SE, Wiman NG, et al. *Drosophila suzukii* flight performance reduced by starvation but not affected by humidity. J Pest Sci. 2018;91:1269–1278.

18.Tait G, Cabianca A, Grassi A, Pfab F, Oppedisano T, Puppato S, et al. *Drosophila suzukii* daily dispersal between distinctly different habitats. Entomol Gen. 2020;40:25–37. handle: http://hdl.handle.net/10449/58915

19.Crawley MJ. The population biology of invaders. Philos Trans R Soc Lond B Biol Sci. 1986;314:711–731.

20.Hengeveld R. Mechanisms of biological invasions. J Biogeogr. 1988;15:819–828.

21.Bernardi D, Andreazza F, Botton M, Baronio CA, Nava DE. Susceptibility and Interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Damaging Strawberry. Neotrop Entomol. 2017;46:1–7. pmid:27389188

22.Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, et al. *Drosophila suzukii* (Diptera: Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. J Integr Pest Manag. 2011;2:G1–G7.

23.Fraimout A, Debat V, Fellous S, Hufbauer RA, Foucaud J, Pudlo P, et al. Deciphering the routes of invasion of *Drosophila suzukii* by means of ABC random forest. Mol Biol Evol. 2017;34:980–996. pmid:28122970

24.Little CM, Little CM, Chapman TW, Hillier NK. Plasticity Is Key to Success of *Drosophila suzukii* (Diptera: Drosophilidae) Invasion. J Insect Sci. 2020;20:5. pmid:32417920

25.Shrader ME. *Drosophila suzukii* (Matsumura) (Diptera: Drosphilidae): Risk Assessment For An Invasive Vinegar Fly In Virginia Vineyards. Ph.D. Dissertation, Virginia Polytechnic Institute and State University. 2017. http://hdl.handle.net/10919/86414

26.Yokomizo H, Possingham HP, Thomas MB, Buckley YM. Managing the impact of invasive species: The value of knowing the density-impact curve. Ecol Appl. 2009;19:376–386. pmid:19323196

27.Pachepsky E, Levine JM. Density dependence slows invader spread in fragmented landscapes. Am Nat. 2011;177:18–28. pmid:21117949

28.Sullivan LL, Li B, Miller TEX, Neubert MG, Shaw AK. Density dependence in demography and dispersal generates fluctuating invasion speeds. Proc Natl Acad Sci U S A. 2017;114:5053–5058. pmid:28442569

29.Golden S, Dukas R. The value of patch-choice copying in fruit flies. PLoS One. 2014;9(11):e112381. pmid:25375776

30.Desharnais R. Advances in Ecological Research Vol 37.: Population Dynamics and Laboratory Ecology. Amsterdam: Elsevier; 2005

31.Wetzel WC, Strong DR. Host selection by an insect herbivore with spatially variable density dependence. Oecologia. 2015;179:777–784. pmid:26120095

32.Rohlfs M, Hoffmeister TS. Spatial aggregation across ephemeral resource patches in insect communities: An adaptive response to natural enemies? Oecologia. 2004;140:654–661. pmid:15232730

33.Sherpa S, Després L. The evolutionary dynamics of biological invasions: A multi-approach perspective. Evol Appl. 2021;14:1463–1484. pmid:34178098

34.Peacock L, Worner S, Sedcole R. Climate variables and their role in site discrimination of invasive insect species distributions. Environ Entomol. 2006;35:958–963.

35.Liebhold AM, Yamanaka T, Roques A, Augustin S, Chown SL, Brockerhoff EG, et al. Plant diversity drives global patterns of insect invasions. Sci Rep. 2018;8:1–5. pmid:30108295

36.Johnson CA, Coutinho RM, Berlin E, Dolphin KE, Heyer J, Kim B, et al. Effects of temperature and resource variation on insect population dynamics: the bordered plant bug as a case study. Funct Ecol. 2016;30:1122–1131. pmid:28824219

37.Shea K, Chesson P. Community ecology theory as a framework for biological invasions. Trends Ecol Evol. 2002;17:170–176.

38.Pyšek P, Jarošík V, Hulme PE, Kühn I, Wild J, Arianoutsou M, et al. Disentangling the role of environmental and human pressures on biological invasions across Europe. Proc Natl Acad Sci U S A. 2010;107:12157–12162. pmid:20534543

39.Bezerra Da Silva CS, Park KR, Blood RA, Walton VM. Intraspecific competition affects the pupation behavior of spotted-wing *Drosophila* (*Drosophila suzukii*). Sci Rep. 2019;9:7775. pmid:31123337

40.Hardin JA, Kraus DA, Burrack HJ. Diet quality mitigates intraspecific larval competition in *Drosophila suzukii*. Entomol Exp Appl. 2015;156:59–65.

41.Kidera H, Takahashi KH. Chemical cues from competitors change the oviposition preference of *Drosophila suzukii*. Entomol Exp Appl. 2020;168:304–310.

42.Shaw B, Brain P, Fountain MT. Reducing *Drosophila suzukii* emergence through inter-species competition. Pest Manag Sci. 2018;74:1466–1471. pmid:29266721

43.Shrader ME, Burrack HJ, Pfeiffer DG. Effects of interspecific larval competition on developmental parameters in nutrient sources between *Drosophila suzukii* (Diptera: Drosophilidae) and *Zaprionus indianus*. J Econ Entomol. 2020;113:230–238. pmid:31742340

44.Hodgson ES. Chemoreception in arthropods. Annu Rev Entomol. 1958;3:19–36.

45.Chandler CK, Legard DE, Dunigan DD. “Strawberry festival” strawberry. HortScience. 2000;35:1366–1367.

46.Van Timmeren S, Isaacs R. *Drosophila suzukii* in Michigan vineyards, and the first report of *Zaprionus indianus* from this region. J Appl Entomol. 2014;138:519–527.

47.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 Manag Sci. 2011. pmid:21898759

48.van Der Linde K. *Zaprionus indianus*: Species identification and taxonomic position. Drosoph Inf Serv. 2010; 93:95–98

49.Andreazza F, Bernardi D, Nava DE, Marangon RB, Botton M, Scheunemann T. Técnica de criação de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). Embrapa Clima Temperado-Boletim de Pesquisa e Desenvolvimento. 2016. http://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAT-2010/4779/1/Bp-023.pdf

50.de Souza MT, de Souza MT, Bernardi D, Rakes M, Vidal HR, Zawadneak MAC. Physicochemical characteristics and superficial damage modulate persimmon infestation by *Drosophila suzukii* (Diptera: Drosophilidae) and *Zaprionus indianus*. Environ Entomol. 2020;49:1290–1299. pmid:33051661

51.Revadi S, Lebreton S, Witzgall P, Anfora G, Dekker T, Becher PG. Sexual behavior of *Drosophila suzukii*. Insects. 2015;6:183–196. pmid:26463074

52.Leslie PH. On the use of matrices in certain population mathematics. Biometrika. 1945;33:183–212. pmid:21006835

53.Gotelli NJ et al. A primer of ecology. 4th ed. Sunderland, MA: Sinauer Associates 2008.

54.Travis CC, Post WM, DeAngelis DL, Perkowski J. Analysis of compensatory Leslie matrix models for competing species. Theor Popul Biol. 1980;18:16–30. pmid:7466670

55.Bernardi D, Andreazza F, Nava D, Baronio C, Botton M. Duplo ataque. Cultivar [internet]. 2015. https://www.alice.cnptia.embrapa.br/bitstream/doc/1032842/1/DuploataquecultivarHF.pdf

56.Lasa R, Tadeo E. Invasive Drosophilid Pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. Fla Entomol. 2015;98:987–988.

57.Justin MR, Iglesias LE, Bonneau P, Liburd OE. Trapping system comparisons for and factors affecting populations of *Drosophila suzukii* and *Zaprionus indianus* in winter-grown strawberry. Pest Manag Sci. 2018;74:2076–2088. pmid:29516620

58.Escobar LI, Ovruski Alderete SM, Kirschbaum DS. Foreign invasive pests *Drosophila suzukii* (Matsamura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) threaten fruit production in northwestern Argentina. Curr Trends Entomol; CTEZS-104.

59.Fartyal RS, Sarswat M, Lhamo N, Sati PC, Asha L. Records of *Zaprionus indianus* and *Drosophila suzukii* indicus as invasive fruit pests from mid valley region of Garhwal Uttarakhand, India. Drosoph Inf Serv. 2014;97:119–123.

60.Duarte LB, Gottschalk MS, Robe LJ. Assemblage of drosophilids (Diptera, Drosophilidae) inhabiting flooded and nonflooded areas in the extreme South of Brazil. Rev Bras Entomol. 2018;62:29–35.

61.Zanuncio-Junior JS, Fornazier MJ, Andreazza F, Culik MP, Mendonça LDP, Oliveira EE, et al. Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in southeastern Brazil. Fla Entomol. 2018;101:522–525.

62.Elsensohn JE, Schal C, Burrack HJ. Plasticity in Oviposition Site Selection Behavior in *Drosophila suzukii* (Diptera: Drosophilidae) in Relation to Adult Density and Host Distribution and Quality. J Econ Entomol. 2021;114:1517–1522. pmid:34114635

63.Takahashi KH, Kimura MT. Intraspecific and interspecific larval interaction in *Drosophila* assessed by integrated fitness measure. Oikos. 2005;111:574–581.

64.Gao HH, Zhai YF, Chen H, Wang YM, Liu Q, Hu QL, et al. Ecological niche difference associated with varied ethanol tolerance between *Drosophila suzukii* and *Drosophila melanogaster* (Diptera: Drosophilidae). Fla Entomol. 2018;498–504.

65.Emiljanowicz LM, Ryan GD, Langille A, Newman J. Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. J Econ Entomol. 2014; 107: 1392–1398. pmid:25195427

66.Jaramillo SL, Mehlferber E, Moore PJ. Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: Drosophilidae). Physiological Entomology. 2015; 40: 2–9.

67.Hoffmann DCS, Wollmann J, Krüger AP, Nunes AM, Bernardi D, Garcia FRM. Biology and fertility life table of *Drosophila suzukii* on artificial diets. Entomol Exp Appl. 2018; 166: 932–936.

68.Krüger AP, Vieira JG, Scheunemann T, Nava DE, Garcia FR. Effects of temperature and relative humidity on mating and survival of sterile *Drosophila suzukii*. J Appl Entomol. 2021; 145: 789–799.

69.Hamby KA, Bellamy D, Chiu JC, Lee JC, Walton VM, Wiman NG, et al. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. J Pest Sci. 2016 89: 605–619.

70.Abrams PA. When does greater mortality increase population size? the long history and diverse mechanisms underlying the hydra effect. Ecol Lett. 2009;12:462–474. pmid:19220393

71.Schröder A, van Leeuwen A, Cameron TC. When less is more: Positive population-level effects of mortality. Trends Ecol Evol. 2014;29:614–624. pmid:25262501

72.Rosa GS, Costa MIS, Corrente JE, Silveira LVA, Godoy WAC. Population dynamics, life stage and ecological modeling in *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae). Neotrop Entomol. 2011;40:181–189. pmid:21584398

73.Botteon VW, Neves JA, Godoy WAC. Functional Response and Matrix Population Model of *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) fed on *Chrysomya putoria* (Wiedemann, 1818) (Diptera: Calliphoridae) as Alternative Prey. Neotrop Entomol. 2017;46:137–143. pmid:27604849

74.Auer TO, Benton R. Sexual circuitry in *Drosophila*. Curr Opin Neurobiol. 2016;38:18–26. pmid:26851712

75.Khallaf MA, Cui R, Weißflog J, Erdogmus M, Svatoš A, Dweck HKM, et al. Large-scale characterization of sex pheromone communication systems in *Drosophila*. Nat Commun. 2021;12:4165. pmid:34230464

76.Keesey IW, Knaden M, Hansson BS. Olfactory Specialization in *Drosophila suzukii* Supports an Ecological Shift in Host Preference from Rotten to Fresh Fruit. J Chem Ecol. 2015;41:121–128. pmid:25618323

77.Döge JS, Oliveira HV, Tidon R. Rapid response to abiotic and biotic factors controls population growth of two invasive drosophilids (Diptera) in the Brazilian Savanna. Biol Invasions. 2015;17:2461–2474.

78.Daehler CC. Performance Comparisons of Co-Occurring Native and Alien Invasive Plants: Implications for Conservation and Restoration. Annu Rev Ecol Evol Syst. 2003;34:183–211.

79.Vilà M, Weiner J. Are invasive plant species better competitors than native plant species? evidence from pair-wise experiments. Oikos. 2004;105:229–238.

80.Sheikh AA, Rehman NZ, Kumar R. Diverse Adaptations in Insects: A Review. J Entomol Zool Stud. 2017;5:343–350.

81.Machado da Silva N, Fantinel C da C, Valente VLS, Valiati VH. Population dynamics of the invasive species *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae) in communities of drosophilids of Porto Alegre city, Southern of Brazil. Neotrop Entomol. 2005;34:363–374.

82.Ferronato P, Woch AL, Soares PL, Bernardi D, Botton M, Andreazza F, et al. A Phylogeographic Approach to the *Drosophila suzukii* (Diptera: Drosophilidae) Invasion in Brazil. J Econ Entomol. 2019;112:425–433. pmid:30383249

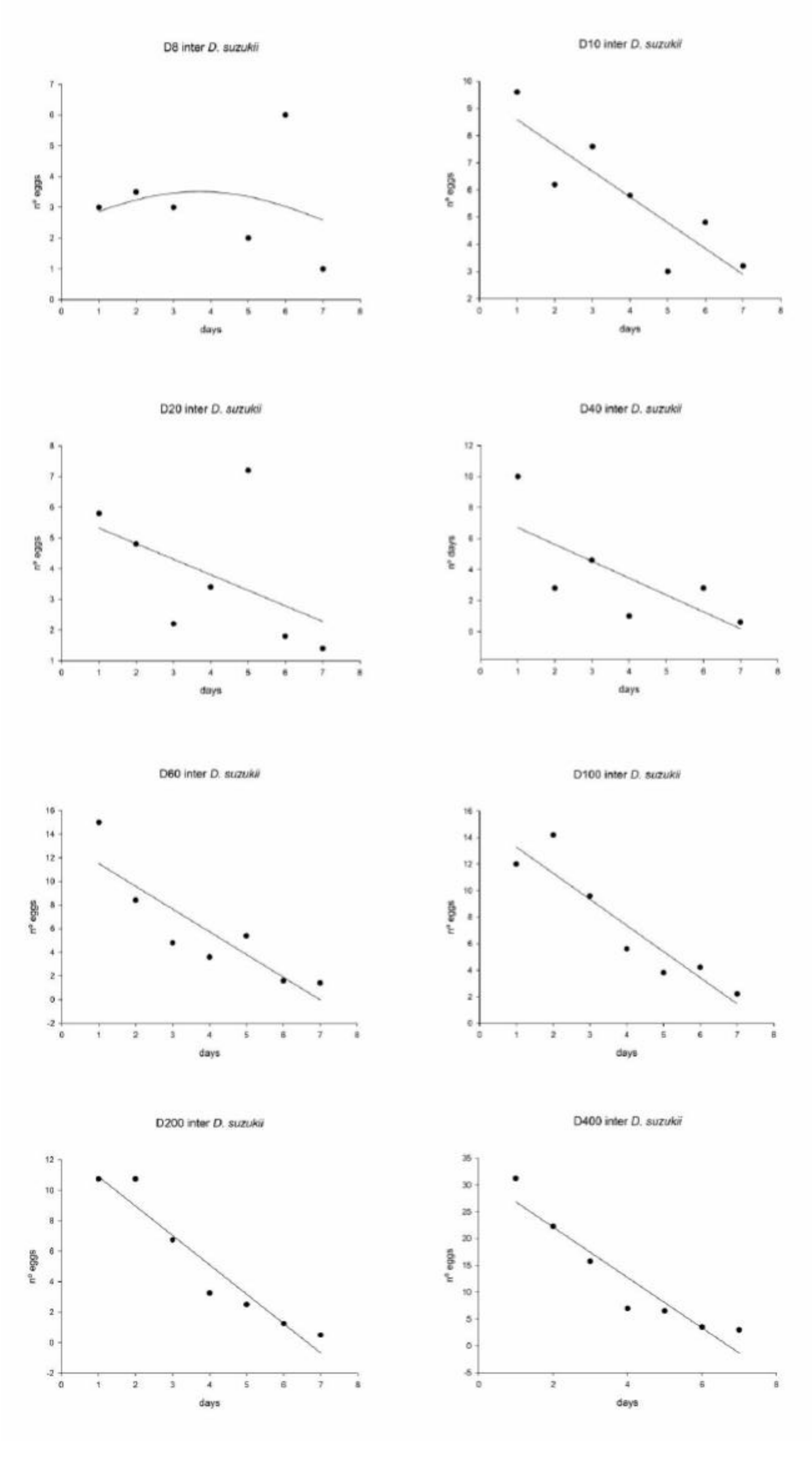
83.Lancaster LT, Morrison G, Fitt RN. Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. Philos Trans R Soc B Biol Sci. 2017;372:20160046. pmid:27920390

84.Åkesson A, Curtsdotter A, Eklöf A, Ebenman B, Norberg J, Barabás G. The importance of species interactions in eco-evolutionary community dynamics under climate change. Nat Commun. 2021;12:4759. pmid:34362916

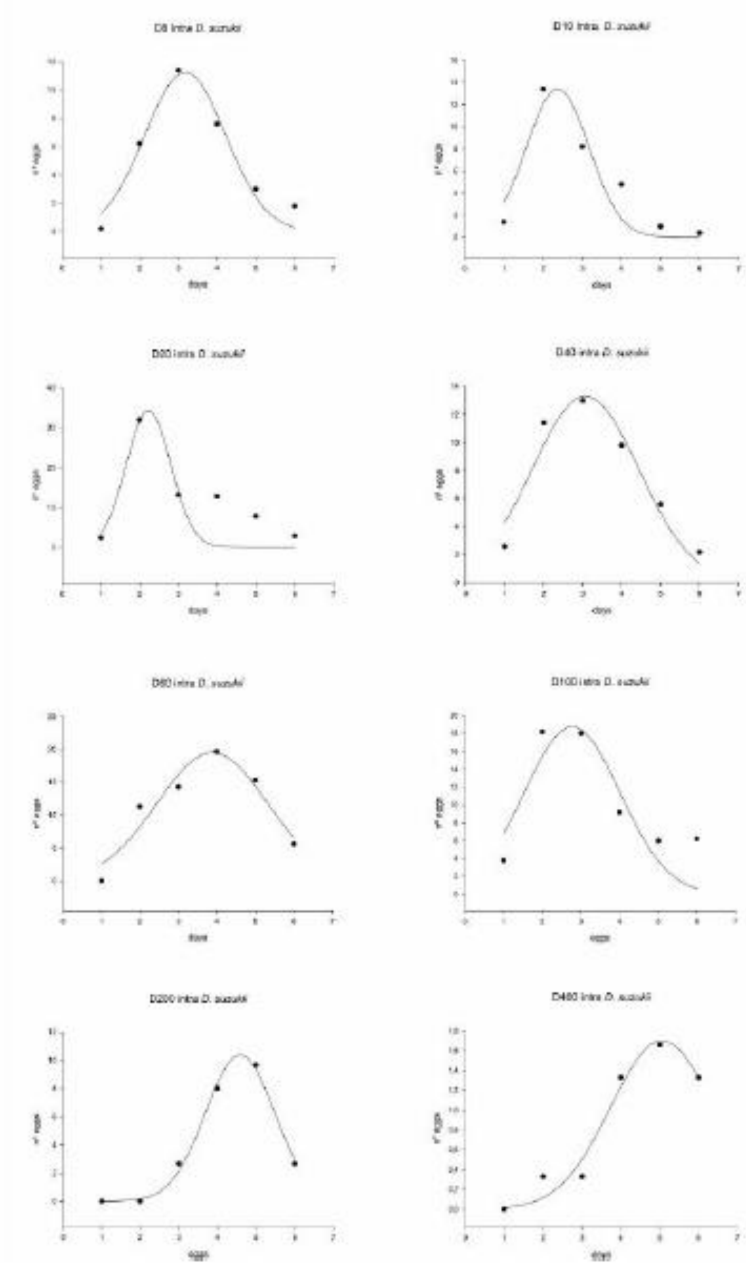
Supplementary Material

S1 Table. Summary of regression analyses for the decrease in the eggs laying by the females of *D. suzukii* in intraspecifc and interspecific density. (shown in S Fig 1 and 2)

Variable	Model	Treatment	Estimated parameters a	b	y_0 or x_0	df_{error}	F	p	R^2
Intraspecific fecundity	f = a*exp(-.5*((x-x0)/b)^2)	D8	11.25 (7.81-14.68)	1.04 (0.67-1.41)	3.17 (2.80-3.54)	3	28.53	0.01	0.97
		D10	13.38 (5.02-21.73)	0.80 (0.19-1.41)	2.37 (1.81-2.92)	3	9.32	0.05	0.92
		D20	34.27 (-4.11-72.66)	0.57 (-0.21-1.36)	2.23 (1.36-3.09)	3	2.44	0.23	0.78
		D40	13.30 (9.40-17.20)	1.37 (0.88-1.87)	3.07 (2.60-3.53)	3	21.04	0.01	0.96
		D60	19.51 (12.87-26.16)	1.43 (0.82-2.03)	3.88 (3.31-4.44)	3	16.47	0.02	0.95
		D100	18.72 (7.01-30.44)	1.24 (0.29-2.19)	2.76 (1.86-3.67)	3	3.69	0.15	0.84
		D200	10.37 (8.92-11.82)	0.89 (0.74-1.04)	4.58 (4.44-4.72)	3	196.71	0.0007	0.99
		D400	1.70 (1.24-2.16)	1.31 (0.73-1.90)	5.05 (4.54-5.57)	3	39.33	0.007	0.98
Interspecific fecundity	f = a*exp(-.5*((x-x0)/b)^2)	D8	3.51 (-1.51-8.55)	4.20 (-16.63-25.04)	3.69 (-4.51-11.90)	3	0.08	0.92	0.22
	f = y0+a*x	D10	-0.95 (-1.57- -0.32)	_	9.54 (6.76-12.3)	5	15.50	0.01	0.86
		D20	-0.507 (-1.52-0.50)	_	5.82 (1.28-10.36)	5	1.64	0.25	0.49
		D40	-1.08 (-2.48-0.31)	_	7.80 (1.67-13.92)	4	4.64	0.09	0.73
		D60	-1.92 (-3.13 - -0.71)	_	13.42 (8.01-18.83)	5	16.67	0.009	0.87
		D100	-1.97 (-2.86 - -1.07)	_	15.25 (11.26-19.25)	5	32.21	0.002	0.93
		D200	-1.92 (-2.56 - -1.29)	_	12.82 (9.97-15.66)	5	60.62	0.0006	0.96
		D400	-4.69 (-6.59 - -2.79)	_	31.53 (23.02-40.04)	5	40.27	0.0014	0.94



S1 Fig1: Peak of fecundity response of *Drosophila suzukii* in interspecif competition during the seven days of evaluation



S1 Fig2. Peak of fecundity response of *Drosophila suzukii* in intraspecific competition during the seven days of evaluation

3 EFFECT OF ENDOPHYTIC INOCULATIONS OF STRAWBERRY ROOTS WITH ENTOMOPATHOGENS IN PEST OF LEAF AND FRUIT PARTS IN DIFFERENT DENSITIES

This chapter was submitted to Pest Management Science: MENDONÇA, LDP; HADDI, K; MORAL. RDA, DELALIBERA IJ, GODOY, WAC (2024) Effect of endophytic inoculations of strawberry roots with entomopathogens in pest of leaf and fruit parts in different densities. *Pest Managment Science*

Abstract

BACKGROUND: Endophytism between entomopathogenic fungi and their host plants has been tested for various leaf-feeding pests, such as the red spider mite *Tetranychus urticae*. In strawberry plants, the effect of this interaction still needs to be explored for fruit-feeding pests, such as the spotted wing drosophila *Drosophila suzukii*. Therefore, strawberry roots were inoculated with *Metarhizum robertsii* and *Beauveria bassiana*. The effects of inoculations on the development and behavior of *D. suzukii* and *T. urticae* were evaluated at high and low infestation densities of these pests.

RESULTS: Both fungal species proved promising for direct control of the two pests. For the Camarosa cultivar, inoculation with *M. robertsii* rendered the plants more attractive to females of *D. suzukii*. The pest *D. suzukii* developed better under the combination of both stresses, plants treated with *B. bassiana* and high density, probably an overcompensation behavior. On the same cultivar, the high density of *T. urticae* for both the fungi treatments showed overcompensation on the development of the pest compared to the control. Inoculation of the San Andreas cultivar with both fungi did not affect the development and behavior of either pest.

CONCLUSION: The endophytic inoculations of strawberries did not prove to be the best way to control *D. suzukii*, due to greater attractiveness and the overcompensation effect. Although endophytic inoculation of strawberries showed potential to control *T. urticae*, the density of the pest in the field needs to be considered for the method's efficacy. Combining control methods with reinoculation should be considered to achieve maximum efficacy.

Keywords: *Tetranychus urticae*; *Drosophila suzukii*; Endophytes; Biological control; Intraspecific competition

3.1 Introdction

Relationships between entomopathogenic fungi and several plant species have been well documented.[1] Such interactions occur when fungi colonize the plant asymptomatically without adverse effects.[2] Because plant-microorganism associations can alter plant physiological processes, they are believed to promote plants' tolerance to different biotic and abiotic stressors.[1] Artificial endophytic inoculation of a plant with entomopathogenic fungi has been proposed as an option for pest control. Successful examples of entomopathogenic fungi with promising effects on plant pests have been frequently reported.[3–5] However, some aspects, including the efficiency of these control techniques under different pest densities, remain to be explored and evaluated before wide adoption in agriculture.

Beauveria and *Metarhizium* are two genera of entomopathogenic fungi known to form symbiotic relationships with plants as endophytes.[1,6] Endophytism between entomopathogenic fungi and their host plant protects the fungi from the direct negative actions of abiotic environmental factors as the fungi develop in the internal tissues of a host plant.[1,7]The varying effects against pests under field conditions, described as a significant constraint on using entomopathogenic fungi as microbial control agents, could be reduced under certain conditions[8,9] Field studies have demonstrated the potential of *B. bassiana* in the management of different pest insects, including *Liriomyza* leafminers in bean leaves,[10] *Aphis gossypii* on cotton plants,[11] and *Sesamia nonagrioides* in sorghum plants.[12] Likewise, species of *Metarhizium* have also been developed and marketed for control of several pests.[12–14]

Strawberry (*Fragaria* × *ananassa*) is among the plants studied for the effect of different genera and species of fungi endophytically[9] on different pests, but these studies have focused mainly on foliar and root-attacking pests, with relatively few on pests of the reproductive parts. The spotted wing drosophila *Drosophila suzukii* is an invasive insect found in different soft- and thin-skinned fruit crops, and is a critical pest of strawberries. *D. suzukii* females have a serrated ovipositor that damages small fruits such as strawberries.[15–18] The red spider mite *Tetranychus urticae* is also a recurrent strawberry pest.[19,20] Its feeding on the basal part of leaves impairs photosynthesis, reducing fruit production.[21–23] Different genera, species, and isolates of entomopathogenic fungi have been tested, mainly through direct exposure, for biological control of *D. suzukii* and *T. urticae*.[24–27] Even though different types of exposure have been explored, indirect exposure by inoculating a fungal endophyte into strawberry plants has not been investigated as a control for *D. suzukii*.

Under Integrated Pest Management (IPM) schemes, different control strategies are tested, searching for effective methods to maintain pests below the economic damage level. However, the control outcome can vary due to different ecological factors, including competition. For instance, intraspecific competition density has been reported as an example of the factors influencing niche expansion in insect species,[28] including *D. suzukii*.[29,30] However, the effect of intraspecific competition density as found in the field on the performance of alternative control techniques has not yet been studied. Furthermore, even though endophytic inoculation of plants has been shown as a promising alternative to pest control, its effectiveness can vary with the fungi and plant cultivars used. In fact, the fungal virulence and the plant's interaction with pests are intimately linked with the pathogen genus, species, and isolates used as well as with the plant cultivar.[9,31]

The present study evaluated the effects of root inoculation of two strawberry cultivars with two selected isolates of entomopathogenic fungi on pests of different plant parts, *D. suzukii* and *T. urticae*, in high and low infestation densities. The fungi tested were *Beauveria bassiana* ESALQ 3323 and *Metarhizium robertsii* ESALQ 1634. Our hypotheses were: (i) Inoculation of endophytic fungi has different control efficacies against foliar and fruit pests, (ii) the effects of fungal inoculation on pest development depend on the plant cultivar, and (iii) the density of the pest infestation can affect the response of plants endophytically treated with fungi.

References

1 Vega FE, The use of fungal entomopathogens as endophytes in biological control: a review. *Mycologia* **110**:4–30 (2018).

2 Hirsch G and Braun U, Communities of parasitic microfungi, in *Fungi in Vegetation Science*, ed. by Winterhoff W. Springer, Dordrecht, pp. 225–250 (1992).

3 Kabaluk JT and Ericsson JD, *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. *Agron J* **99**:1377–1381 (2007).

4 Powell WA, Klingeman WE, Ownley BH and Gwinn KD, Evidence of endophytic *Beauveria bassiana* in seed-treated tomato plants acting as a systemic entomopathogen to larval *Helicoverpa zea* (Lepidoptera: Noctuidae). *J Entomol Sci* **44**:391–396 (2009).

5 Gurulingappa P, Sword GA, Murdoch G and McGee PA, Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when *in planta*. *Biol Control* **55**:34–41 (2010).

6 Greenfield M, Gómez-Jiménez MI, Ortiz V, Vega FE, Kramer M and Parsa S, *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. *Biol Control* **95**:40–48 (2016).

7 Jaber LR and Ownley BH, Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? *Biol Control* **116**:36–45 (2018).

8 Castro T, Eilenberg J and Delalibera I, Exploring virulence of new and less studied species of *Metarhizium* spp. from Brazil for two-spotted spider mite control. *Exp Appl Acarol* **74**:139–146 (2018).

9 Canassa F, D'Alessandro CP, Sousa SB, Demétrio CGB, Meyling NV, Klingen I *et al.*, Fungal isolate and crop cultivar influence the beneficial effects of root inoculation with entomopathogenic fungi in strawberry. *Pest Manag Sci* **76**:1472–1482 (2020).

10 Gathage JW, Lagat ZO, Fiaboe KKM, Akutse KS, Ekesi S and Maniania NK, Prospects of fungal endophytes in the control of *Liriomyza leafminer* flies in common bean *Phaseolus vulgaris* under field conditions. *BioControl* **61**:741–753 (2016).

11 Lopez DC, Zhu-Salzman K, Ek-Ramos MJ and Sword GA, The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. *PLoS One* **9**(8):e103891 (2014).

12 Mantzoukas S, Chondrogiannis C and Grammatikopoulos G, Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer *Sesamia nonagrioide*s. *Entomol Exp Appl* **154**:78–87 (2015).

13 Akutse KS, Subramanian S, Maniania NK, Dubois T and Ekesi S, Biopesticide research and product development in Africa for sustainable agriculture and food security – Experiences From the International Centre of Insect Physiology and Ecology (*icipe*). *Front Sustain Food Syst* **4**:1–14 (2020).

14 Bamisile BS, Akutse KS, Siddiqui JA and Xu Y, Model Application of Entomopathogenic Fungi as Alternatives to Chemical Pesticides: Prospects, Challenges, and Insights for Next-Generation Sustainable Agriculture. *Front Plant Sci* **12**:741804 (2021).

15 Atallah J, Teixeira L, Salazar R, Zaragoza G and Kopp A, The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proc R Soc B Biol Sci* **281**:20132840 (2014).

16 Lee JC, Bruck DJ, Dreves AJ, Ioriatti C, Vogt H and Baufeld P, In focus: spotted wing drosophila, *Drosophila suzukii*, across perspectives. *Pest Manag Sci* **67**:1349–1351 (2011).

17 Lee JC, Bruck DJ, Curry H, Edwards D, Haviland DR, Van Steenwyk RA *et al.*, The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Manag Sci* **67**:1358–1367 (2011).

18 Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM *et al.*, Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. *J Pest Sci* **88**:469–494 (2015).

19 Castilho RC, Duarte VS, de Moraes GJ, Westrum K, Trandem N, Rocha LCD *et al.*, Two-spotted spider mite and its natural enemies on strawberry grown as protected and unprotected crops in Norway and Brazil. *Exp Appl Acarol* **66**:509–528 (2015).

20 Raworth DA, An economic threshold function for the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on strawberries. *Can Entomol* **118**:9–16 (1986).

21 Sances FV., Wyman JA and Ting IP, Physiological responses to spider mite infestation on strawberries. *Environ Entomol* **8**:711–714 (1979).

22 Johnson WT and Lyon HH, *Insects that Feed on Trees and Shrubs*, Second Edition, Revised. Cornell University Press, Ithaca, New York (1988).

23 Nyoike TW and Liburd OE, Effect of *Tetranychus urticae* (Acari: Tetranychidae), on marketable yields of field-grown strawberries in north-central Florida. *J Econ Entomol* **106**:1757–1766 (2013).

24 Cahenzli F, Strack T and Daniel C, Screening of 25 different natural crop protection products against *Drosophila suzukii*. *J Appl Entomol* **142**:563–577 (2018).

25 Cuthbertson AGS and Audsley N, Further screening of entomopathogenic fungi and nematodes as control agents for *Drosophila suzukii*. *Insects* **7**:24 (2016).

26 Dogan YO, Hazir S, Yildiz A, Butt TM and Cakmak I, Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). *Biol Control* **111**:6–12 (2017).

27 Maniania NK, Bugeme DM, Wekesa VW, Delalibera I and Knapp M, Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. *Exp Appl Acarol* **46**:259–274 (2008).

28 Bolnick DI, Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. *Nature* **410**:463 (2001).

29 Bezerra Da Silva CS, Park KR, Blood RA and Walton VM, Intraspecific Competition Affects the Pupation Behavior of Spotted-Wing Drosophila (*Drosophila suzukii*). *Sci Rep* **9**:7775 (2019).

30 Mendonça LDP, Haddi K and Godoy WAC, Effects of co-occurrence and intra- and interspecific interactions between *Drosophila suzukii* and *Zaprionus indianus*. *PLoS One* **18**:e0281806 (2023).

31 Valero-Jiménez CA, Debets AJM, van Kan JAL, Schoustra SE, Takken W, Zwaan BJ *et al.*, Natural variation in virulence of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes. *Malar J* **13**:479 (2014).

32 Andreazza F, Bernardi D, Marangon RB, Scheunemann T, Botton M and Nava DE, Técnica de criação de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). Embrapa Clima Temperado, *Bol Pesqui Desenvolv* **240**:1–24 (2016).

33 Mendonca LDP, Oliveira EE, Andreazza F, Rezende SM, Faroni LRDA, Guedes RNC *et al.*, Host Potential and Adaptive Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Barbados Cherries. *J Econ Entomol* **112**:3002–3006 (2019).

34 Oliveira DGP, Pauli G, Mascarin GM and Delalibera I, A protocol for determination of conidial viability of

the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. *J Microbiol Methods* **119**:44–52 (2015).

35 Alves SB, Controle microbiano de insetos (1986).

36 Alnajjar G, Drummond FA and Groden E, Laboratory and Field Susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to Entomopathogenic Fungal Mycoses. *J Agric Urban Entomol* **33**:111–132 (2017).

37 Ibouh K, Oreste M, Bubici G, Tarasco E, Rossi Stacconi MV, Ioriatti C *et al.*, Biological control of *Drosophila suzukii:* Efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. *Crop Prot* **125**:104897 (2019).

38 Zaman S, Rasul A, Khan AG, Ali Q, Anwar T, Faisal M *et al.*, Effect of abiotic factors on the efficacy of *Beauveria bassiana* and diatomaceous earth against *Rhyzopertha dominica* (F.): Efficacy of bio-pesticides against *R. dominica* under different abiotic factors. *J Appl Biol Sci* **14**:98–112 (2020).

39 Braga GUL, Flint SD, Miller CD, Anderson AJ and Roberts DW, Both Solar UVA and UVB Radiation Impair Conidial Culturability and Delay Germination in the Entomopathogenic Fungus *Metarhizium anisopliae*. *Photochem Photobiol* **74**:734 (2001).

40 Fernandes ÉKK, Rangel DEN, Braga GUL and Roberts DW, Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. *Curr Genet* **61**:427–440 (2015).

41 Canassa F, D'Alessandro CP, Sousa SB, Demétrio CGB, Meyling NV, Klingen I *et al.*, Fungal isolate and crop cultivar influence the beneficial effects of root inoculation with entomopathogenic fungi in strawberry. *Pest Manag Sci* **76**:1472–1482 (2020).

42 Manoussopoulos Y, Mantzoukas S, Lagogiannis I, Goudoudaki S and Kambouris M, Effects of Three Strawberry Entomopathogenic Fungi on the Prefeeding Behavior of the Aphid *Myzus persicae*. *J Insect Behav* **32**:99–108 (2019).

43 Canassa F, Tall S, Moral RA, Lara IAR, Delalibera I and Meyling NV, Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite populations and behavior of predatory mites. *Biol Control* **132**:199–208 (2019).

44 Dash CK, Bamisile BS, Keppanan R, Qasim M, Lin Y, Islam SU *et al.*, Endophytic entomopathogenic fungi enhance the growth of *Phaseolus vulgaris* L. (Fabaceae) and negatively affect the development and reproduction of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Microb Pathog* **125**:385–392 (2018).

45 Rasool S, Cárdenas PD, Pattison DI, Jensen B and Meyling NV, Isolate-Specific Effect of Entomopathogenic Endophytic Fungi on Population Growth of Two-Spotted Spider Mite (*Tetranychus urticae* Koch) and Levels of Steroidal Glycoalkaloids in Tomato. *J Chem Ecol* **47**:476–488 (2021).

46 Bhagyashree SN, Suroshe SS and Kalia VK, Effect of endophytic colonisation of entomopathogenic *Beauveria bassiana* (Balsamo) on two spotted spider mite *Tetranychus urticae* (Koch) in tomato. *Indian J Entomol* **82**:716–719 (2020).

47 Posada F, Aime MC, Peterson SW, Rehner SA and Vega FE, Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycol Res* **111**:748–757 (2007).

48 Flores Cantillano RF and Silva MM, Manuseio pós-colheita de morangos. Embrapa Clima Temperado, *Documento* **318**:1–36 (2010).

49 Luckey TD, Insecticide hormoligosis. *J Econ Entomol* **61**:7–12 (1968).

50 Guedes RNC, Magalhães LC and Cosme LV, Stimulatory sublethal response of a generalist predator to permethrin: Hormesis, hormoligosis, or homeostatic regulation? *J Econ Entomol* **102**:170–176 (2009).

51 Rotem KA and Agrawal AA, Density dependent population growth of the two-spotted spider mite, *Tetranychus urticae*, on the host plant *Leonurus cardiaca*. *Oikos* **103**:559–565 (2003).

52 Santos RSS dos, Ocorrência de *Drosophila suzukii* (Matsumura, 1931), (Diptera: Drosophilidae) atacando frutos de morango no Brasil. Embrapa Uva e Vinho, *Comunicado Técnico* **159**:1–4 (2014).

53 Pedigo LP, Hutchins SH and Higley LG, Economic injury levels in theory and practice. *Annu Rev Entomol* **31**:341–368 (1986).

54 Shrivastava G, Ownley BH, Augé RM, Toler H, Dee M, Vu A *et al.*, Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. *Symbiosis* **65**:65–74 (2015).

55 Muvea AM, Meyhöfer R, Maniania NK, Poehling HM, Ekesi S and Subramanian S, Behavioral responses of *Thrips tabaci* Lindeman to endophyte-inoculated onion plants. *J Pest Sci* **88**:555–562 (2015).

56 Sword GA, Tessnow A and Ek-Ramos MJ, Endophytic fungi alter sucking bug responses to cotton reproductive structures. *Insect Sci* **24**:1003–1014 (2017).

4 APPARENT COMPETITION INVESTIGATION OF *Drosophila suzukii* and *Zaprionus indianus* INTERACTING WITH ITS COMMON NATURAL ENEMY *Metarhizium spp*. UNDER LABORATORY CONDITIONS

This chapter will be submitted to the Journal of Economic Entomology: MENDONÇA, LDP; HADDI, K; GODOY, WAC (2024) Apparent competition investigation of *Drosophila suzukii* and *Zaprionus indianus* interacting with its common natural enemy *Metarhizium spp*. under laboratory conditions.

Abstract

Drosophila suzukii is a polyphagous pest found in the soil during the last larval phase to pupate on this substrate. When in the soil, *D. suzukii* enters into contact with the fauna, including the secondary pest *Z. indianus*, in the pupal phase. Also, there is a diverse range of natural enemies for the pests on the soil. The presence of both pests in the same environment, sharing a natural enemy, may characterize apparent competition. This study evaluated the possibility of apparent competition on laboratory assays with the *D. suzukii* and *Z. indianus* sharing the natural enemy *Metarhizium spp*. The bioassay was divided into two parts. First, a screening bioassay was performed to select the most virulent fungi by direct contact with the pest. One strain of each species of *Metarhizum* was then tested to evaluate the indirect effect of apparent competition. No substantial evidence of apparent competition was observed. However, a marginal effect of the treatments *M. humberri*, *M. brunei,* and *M. anisopliae* was observed with higher mortalities of *D. suzukii* when compared to *Z. indianus* exposed to the same natural enemy. Identifying apparent competition can be critical to anticipating the loss in the field. The knowledge of this interaction emphasizes the need to understand ecological interactions, mainly when natural enemies decrease their interaction strength in response to sharing between hosts or prey. This understanding is crucial for developing sustainable and effective pest control strategies beyond targeting individual pest species.

Keywords: *Drosophila suzukii, Zaprionus indianus*, Apparent competition, interaction

4.1 Introduction

Drosophila suzukii is a species of Drosophilidae first identified in Japan [1]. Its arrival to the West was identified in Hawaii in 1980 [2], but the concerns started with the first identification of the species occurring in strawberry fields in California-USA [3,4]. Since then, it has spread worldwide [5–7]. Different from other *Drosophila* species, *D. suzukii* can infest healthy fruits with thin skin, such as strawberries, grapes, blueberries, and others [4,5,8]. Its biological cycle occurs in approximately 8-10 days at around 25-26° C, with three larval instars [1,4]. The behavior of females showed the oviposition pattern in single clutches on fresh fruits [9,10].

Drosophila suzuki is an insect with polyphagous habits, which can occur in different fruits [11]. Because of its polyphagous habits, it can co-occur with other species that eventually use the same food source. One of the co-occurring species is *Zaprionus indianus* [12–14]. Commonly known as the African fig fly, *Zaprionus indianus* (Gupta, 1970) has an African origin and was first detected in America in 1999 in São Paulo - Brazil [15]. Like *D. suzukii, Z. indianus* is a polyphagous pest known to be a pest of more than 80 plant species [16]. It is a primary pest of fig crops but is mainly known for attacking and feeding on fruits with the presence of damage that allows their oviposition, such as decomposing fruits or with the previous presence of other pests [17,18]. In this way, as a secondary pest in most fruits, *Z. indianus* can compete for food and space with *D. suzukii* [12].

At the beginning of studies, it was believed that *D. suzukii* pupae occurred mainly in fruits [1,5]. As more became known about the pest, it was possible to notice the occurrence of *D. suzukii* pupae in the soil [19,20]. Studies have shown that intraspecific competition can affect the pupation behavior of *D. suzukii*, with larvae migrating to less crowded environments to escape from competition [21]. Many factors can influence the pupation behavior of *D. suzukii*, such as abiotic effects, like humidity and temperature, the characteristics of the substrate that the pupae develop in, and the presence of competitors [21,22]. The intraspecific competition can be a reason for the new finds of the pupa of *D. suzukii* on soil despite the initial research showing the presence of pupal mainly on the fruits.

Like other fruit flies, *Z. indianus* pupae are mainly found on soil [23]. The pupation site can influence the exposure to biological control agents [24]. On soil, the pupae exposure to biological control agents is higher than the exposure of larvae on fruits [19]. Many potential biological control agents have shown the possibility of controlling *D. suzukii* and *Z. indianus,* including predators like ants and spiders, parasitoids, entomopathogenic fungi, and nematodes [19,25–27]. Many of the options of natural enemies for both prey species can naturally occur in soil, such as microbial diversity [28], with the highlight of entomopathogenic fungi [29,30].

When larvae of both species experience the last larval instar, they go to the soil to pupate and can match the fauna of other species occurring there, including potential natural enemies [19]. The presence of both pests in an environment with biological control agents, such as *Metarhizium* spp., allows apparent competition. Apparent competition is a negative interaction between two species that share the same natural enemy [31,32]. The apparent competition has been investigated as a structuring force in insect communities, possibly impacting biological control [33–35]. The indirect interaction mediated by shared natural enemies may explain future field pathogenicity rates and herbivore abundances [36,37]. This prevision of the interaction is essential for landscape planning, invasion biology, and biological control [36].

As the immatures of both species on the soil are not competing for resources or space, the observation of the reduction of one of the species would be a response of a common natural enemy in the environment growing in number by the abundance of its prey [32,34]. The evidence of apparent competition occurring where the coexistence of *D. suzukii* and *Z. indianus* sharing the same natural enemy would allow us to infer that in one of the possible scenarios of unbalanced interactions, only one species survives. [34]. *Drosophila suzukii* is a primary pest, while *Z. indianus* is a secondary pest [1,15]. Therefore, in a scenario where apparent competition occurs, leading to the existence of only the primary pest, it may be worrying for the target crops of *D. suzukii.*

Thus, the present study aimed to evaluate the apparent competition between the fruit fly species *D. suzukii* and *Z. indianus* and its' natural enemy, *Metarhizium spp.* Then, different species and strains of *Metarhizium spp.* were tested for direct effect on both pests. Then, one strain of each species was selected to assess the apparent competition. Our hypotheses were: (i) The entomopathogenic fungi *Metarhizium spp.* have a direct effect of control on *D. suzukii* and *Z. indianus* (ii) One of the selected strains of *Metarhizium spp.* has an indirect effect on both pest *D. suzukii* and *Z. indianus* resulting in apparent competition.

References

1. Kanzawa T. Studies on *Drosophila suzukii* Mats. Stud *Drosoph suzukii* Mats. 1939.
2. Kaneshiro KY. *Drosophila* (Sophophora) *suzukii (*Matsumura). Proc Hawaiian Entomolo Soc. 1983; 179.

3. Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, *et al. Drosophila suzukii* (Diptera : Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. J Integr Pest Manag. 2011;2. doi:10.1603/IPM10010

4. Lee JC, Bruck DJ, Dreves AJ, Ioriatti C, Vogt H, Baufeld P. In Focus : Spotted wing drosophila , *Drosophila suzukii*, across perspectives. Pest Manag Sci. 2011; 1349–1351. doi:10.1002/ps.2271

5. Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, *et al.* Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci (2004). 2015;88: 469–494. doi:10.1007/s10340-015-0681-z

6. 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. Bull Insectology. 2012; 149–160.

7. Zanuncio-Junior JS, Fornazier MJ, Andreazza F, Culik MP, Mendonça LDP, Oliveira EE, *et al.* Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in Southeastern Brazil. Florida Entomol. 2018;101: 522–525. doi:10.1653/024.101.0328

8. Mendonca LDP, Oliveira EE, Andreazza F, Rezende SM, Faroni LRDA, Guedes RNC, *et al.* Host Potential and Adaptive Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Barbados Cherries. J Econ Entomol. 2019;112: 3002–3006. doi:10.1093/jee/toz195

9. Mitsui H, Takahashi KH, Kimura MT. Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. Popul Ecol. 2006;48: 233–237. doi:10.1007/s10144-006-0260-5

10. Kienzle R, Groß LB, Caughman S, Rohlfs M. Resource use by individual *Drosophila suzukii* reveals a flexible preference for oviposition into healthy fruits. Sci Rep. 2020. doi:10.1038/s41598-020-59595-y

11. Minkov P, Georgiev D, Palagacheva N, Dzhuvinov V. Monitoring of spotted wing drosophila in raspberries and blackberries in troyan region, Bulgaria. Bulg J Agric Sci. 2018;24: 670–673.

12. Mendonça LDP, Haddi K, Godoy WAC. Effects of co-occurrence and intra- and interspecific interactions between *Drosophila suzukii* and *Zaprionus indianus*. PLoS One. 2023;18: 1–20. doi:10.1371/journal.pone.0281806

13. Escobar LI, Ovruski SM, Kirschbaum DS. Foreign invasive pests *Drosophila suzukii* (Matsamura) and *Zaprionus indianus* Gupta (Diptera : Drosophilidae) threaten fruit production in northwestern Agertina. Drosoph Inf Serv. 2018.

14. Van Timmeren S, Isaacs R. *Drosophila suzukii* in Michigan vineyards, and the first report of *Zaprionus indianus* from this region. J Appl Entomol. 2014;138: 519–527. doi:10.1111/jen.12113

15. van der Linde K, Steck GJ, Hibbard K, Birdsley JS, Alonso LM, Houle D. First Records of *Zaprionus Indianus* (Diptera: Drosophilidae), a Pest Species on Commercial Fruits From Panama and the United States of America. Florida Entomol. 2006;89: 402–404. doi:10.1653/0015-4040(2006)89[402:frozid]2.0.co;2

16. Bragard C, Baptista P, Chatzivassiliou E, Di Serio F, Gonthier P, Jaques Miret JA, *et al.* Pest categorisation of *Zaprionus indianus*. EFSA J. 2022;20. doi:10.2903/j.efsa.2022.7144

17. Stein CP. Aspectos biológicos da mosca do figo, *Zaprionus indianus* Gupta, 1970. Entomotropica. 2003;18.

18. VILELA CR. Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the neotropical region? Drosoph Inf Serv. 1999;82: 37–39.

19. Woltz JM, Lee JC. Pupation behavior and larval and pupal biocontrol of *Drosophila suzukii* in the field. Biol Control. 2017;110: 62–69. doi:10.1016/j.biocontrol.2017.04.007

20. Ballman ES, Collins JA, Drummond FA. Pupation Behavior and Predation on *Drosophila suzukii* (Diptera: Drosophilidae) Pupae in Maine Wild Blueberry Fields. J Econ Entomol. 2017;110: 2308–2317.

doi:10.1093/jee/tox233

21. Bezerra Da Silva CS, Park KR, Blood RA, Walton VM. Intraspecific Competition Affects the Pupation Behavior of Spotted-Wing Drosophila (*Drosophila suzukii*). Sci Rep. 2019;9: 1–9. doi:10.1038/s41598-019-44248-6
22. Tochen S, Woltz JM, Dalton DT, Lee JC, Wiman NG, Walton VM. Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. J Appl Entomol. 2016;140: 47–57. doi:10.1111/jen.12247
23. Christenson, L.D; Foote RH. Biology of fruit fly. Annu Rev Entomol. 1960;5: 171–192.
24. Hodgson PJ, Sivinski J, Quintero G, Aluja M. Depth of pupation and survival of fruit fly (Anastrepha spp.: Tephritidae) pupae in a range of agricultural habitats. Enviromental Entomol. 1998;27.
25. Ibouh K, Oreste M, Bubici G, Tarasco E, Rossi Stacconi MV, Ioriatti C, *et al.* Biological control of *Drosophila suzukii*: Efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. Crop Prot. 2019;125: 104897. doi:10.1016/j.cropro.2019.104897
26. Svedese VM, da Silva APAP, Lopes RS, dos Santos JF, Lima EÁLA. Ação de fungos entomopatogênicos em larvas e adultos da mosca do figo *Zaprionus indianus* (Diptera: Drosophilidae). Cienc Rural. 2012;42: 1916–1922. doi:10.1590/S0103-84782012001100002
27. Marchiori CH, Silva CG. First occurrence of parasitoid *Spalangia endius* (Walker) (Hymenoptera: Pteromalidae) in pupae of *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in Brazil. Braz J Biol. 2003;63: 361–362. doi:10.1590/S1519-69842003000200022
28. Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, *et al.* A communal catalogue reveals Earth's multiscale microbial diversity. Nature. 2017;551: 457–463. doi:10.1038/nature24621
29. Meyling NV., Eilenberg J. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric Ecosyst Environ. 2006;113: 336–341. doi:10.1016/j.agee.2005.10.011
30. Rocha LFN, Inglis PW, Humber RA, Kipnis A, Luz C. Occurrence of *Metarhizium spp.* in central brazilian soils. J Basic Microbiol. 2013;53: 251–259.
31. Holt RD, Bonsall MB. Apparent Competition. Annu Rev Ecol Evol Syst. 2017;48: 447–471. doi:10.1146/annurev-ecolsys-110316-022628
32. Bonsall MB, Hassell MP. Apparent competition structures ecological assemblages. Nature. 1997;388: 371–373. doi:10.1038/41084
33. Jefries MJ, Lawton JH. Enemy free space and the structure of ecological communities. Biol J Linn Soc. 1984;23: 269–286. doi:10.1111/j.1095-8312.1984.tb00145.x
34. Holt RD, Lawtont JH. Apparent competition and enemy-free space in insect host-parasitoid communities. Am Nat. 1993;142: 623–645.
35. Holt RD, Lawton JH. The ecological consequences of shared natural enemies. Annu Rev Ecol Syst. 1994;25: 495–520. doi:10.1146/annurev.es.25.110194.002431
36. Frost CM, Peralta G, Rand TA, Didham RK, Varsani A, Tylianakis JM. Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. Nat Commun. 2016;7: 1–12. doi:10.1038/ncomms12644
37. Meyling N V., Hajek AE. Principles from community and metapopulation ecology: Application to fungal entomopathogens. BioControl. 2010;55: 39–54. doi:10.1007/s10526-009-9246-5
38. Frank Van Veen FJ, Morris RJ, Godfray HC. Apparent competition, quantitative food webs and structure of

phytophagous insect communities. Annu Rev Entomol. 2006;51.

39. Muller CB, Godfray HCJ. Apparent Competition between Two Aphid Species. J Anim Ecol. 1997;66: 57. doi:10.2307/5964
40. Morris RJ, Lewis OT, Godfray HCJ. Experimental evidence for apparent competition in a tropical forest food web. Nature. 2004;428: 310–313. doi:10.1038/nature02394
41. González-Fernández JJ, de la Peña F, Hormaza JI, Boyero JR, Vela JM, Wong E, *et al.* Alternative food improves the combined effect of an omnivore and a predator on biological pest control. A case study in avocado orchards. Bull Entomol Res. 2009;99: 433–444.
42. Janssen A, Pallini A, Venzon M, Sabelis MW. Behavior and indirect interactions in food webs of plant-inhabiting arthropods. Ecology and evolution of the acari: proceedings of the third symposium of the european association of acarologists. 1999. pp. 231–249.
43. Pimm SL; Lawton JH; Cohen JE. Food web patterns and their consequences. Nature. 1991;350: 669–679.
44. Cuthbertson AGS, Audsley N. Further screening of entomopathogenic fungi and nematodes as control agents for *Drosophila suzukii.* Insects. 2016;7. doi:10.3390/insects7020024
45. Gutierrez-Palomares VM, Paulino-Alonso L, Gutierrez JZ, Alatorre-Rosas R.. Pathogenicity and virulence of *Isaria javanica, Metarhizium anisopliae* and *Beauveria bassiana* strains for control of *Drosophila suzukii* (Matsumura). Southwest Entomol. 2021;46: 853–860.
46. Silva APAP. Infectividade de *Metarhizium anisopliae* à *Zaprionus indianus* (mosca-do-figo) sob condições de laboratório. 2007.
47. Kaser JM, Ode PJ. Hidden risks and benefits of natural enemy-mediated indirect effects. Curr Opin Insect Sci. 2016;14: 105–111. doi:10.1016/j.cois.2016.02.004
48. Wootton JT. The nature and consequence of indirect effects in ecological communities. Annu Rev Ecol Syst. 1994;25: 443–466.
49. Lotka AJ. Elements of physical biology. 1925.
50. Van Veen FJF, Müller CB, Pell JK, Godfray HCJ. Food web structure of three guilds of natural enemies: Predators, parasitoids and pathogens of aphids. J Anim Ecol. 2008;77: 191–200. doi:10.1111/j.1365-2656.2007.01325.x
51. Pope T, Croxson E, Pell JK, Godfray HCJ, Müller CB. Apparent competition between two species of aphid via the fungal pathogen *Erynia neoaphidis* and its interaction with the aphid parasitoid *Aphidius ervi.* Ecol Entomol. 2002;27: 196–203. doi:10.1046/j.1365-2311.2002.00397.x
52. Jonsson M, Kaartinen R, Straub CS. Relationships between natural enemy diversity and biological control. Curr Opin Insect Sci. 2017;20: 1–6. doi:10.1016/j.cois.2017.01.001
53. Holt DR. Predation, apparent competition and the structure of prey communities. Theor Popul Biol. 1977;229: 197–229.
54. Jaworski CC, Chailleux A, Bearez P, Desneux N. Apparent competition between major pests reduces pest population densities on tomato crop, but not yield loss. J Pest Sci (2004). 2015;88: 793–803. doi:10.1007/s10340-015-0698-3
55. Chailleux A, Mohl EK, Teixeira Alves M, Messelink GJ, Desneux N. Natural enemy-mediated indirect interactions among prey species: Potential for enhancing biocontrol services in agroecosystems. Pest Manag Sci. 2014;70: 1769–1779. doi:10.1002/ps.3916

5 REMARKS

The thesis highlights the importance of studying the different forms of competition in an invasive pest and its competitor, demonstrating the importance of the study for its management tactics. In the first chapter, field collection proved the co-occurence of the two invasive species *D. suzukii* and *Z. indianus*. Laboratory assays were conducted to understand the effects of intraspecific and interspecific competition on the biological parameters of the pest, such as development and fecundity. Densities of interspecific competition showed *Z. indianus* more fecund than *D. suzukii*. Leslie Matrix projections indicated practically the same dynamics at intraspecific and interspecific densities for *D. suzukii*. The oviposition behavior of *D. suzukii* did not show a preference for diets previously infested or not with either conspecific or heterospecific eggs at different densities. In this way, it is possible to understand that for the pest *D. suzukii*, the presence of the competitor *Z. indianus* does not affect the oviposition behavior and fecundity. The development of *D. suzukii* kept the same pattern when alone with its own species or with a competing species. But, for *Z. indianus*, the presence of competing specie produced more offspring which can explain the high densities of *Z. indianus* in the field.

In the second chapter, it was studied the effect of endophytic inoculation of entomopathogenic fungi on strawberry plants at different densities of the pests. Two strawberry cultivars, two genders of entomopathogenic fungi, and two pests were tested in the chapter. The fungi used on the bioassay, *B. bassiana*, and *M. robertsii* were shown to be efficient for direct control of the pests. The endophytic treatment with entomopathogenic fungi did not show to be an efficient option for biological control in the studied conditions once the Camarosa cultivar was more attractive for *D. suzukii* when treated with *M. robertsii* ESALQ 1634 and an overcompensation effect was observed for both pests on Camarosa cultivar on a high density of the pest. The San Andreas cultivar did not show to affect the development of both pests. In this way, the endophytic treatment as an option for biological control was dependent on the cultivar treated. Also, the density of the pest can be decisive for the efficiency of the treatment since the combination of two stressful factors, instead of controlling the pest, proved to be beneficial.

The third chapter investigated the apparent competition between the pests *D. suzukii* and *Z. indianus* and its common natural enemy, *Metarhizium* spp. A screening bioassay was performed to select the most virulent isolate of each species of *Metarhizium* that was going to be tested for indirect interaction. Once the isolates were chosen, an indirect effect of apparent competition was investigated. Although solid evidence of apparent competition was not noticed, on the treatments of *M. humberri*, *M. brunei*, and *M. anisopliae* as a natural enemy, it was possible to observe a marginal effect with higher numbers of *Z. indianus* than *D. suzukii* after the exposure.

The developments of this thesis have direct implications for ecology and agriculture by showing the importance of understanding the impact of different competition interactions between invasive pests on the behavior and development of the individuals and if the interactions may be transient or permanent. Ecological matters, such as investigating the effects of density competition, showed importance in the context of integrated pest management (IPM). A better understanding of these interactions is essential for developing more adequate techniques for the control of *D. suzukii* and its co-occurring pests*, Z. indianus* and *T. urticae*.