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Fruit and microbial cues in the behavioural ecology and management of *Drosophila suzukii*

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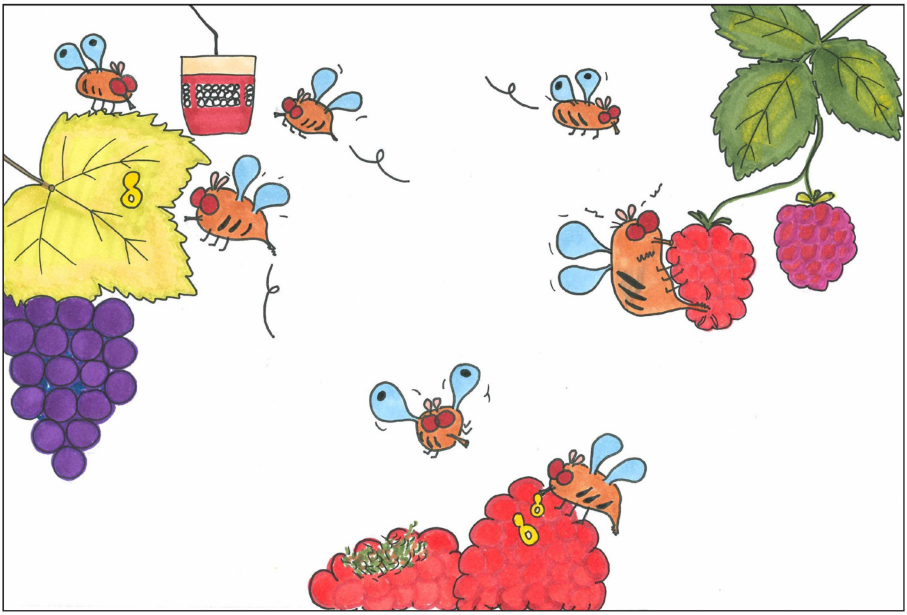

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Alnarp

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Abstract

Investigating the factors that determine the behaviour of new pests is essential for understanding, predicting and managing their impact on natural and agricultural ecosystems.

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae), is a worldwide spreading polyphagous pest of soft fruit and berries. *Drosophila suzukii* is capable to oviposit and develop in ripening fruit, which represents an ecological host shift relative to *Drosophila* species that prefer overripe fruit. Notably, *D. suzukii* lives in close association with yeasts, like saprophagous *Drosophila* flies, but the ecological relevance of this association is insufficiently understood.

Chemical cues are important when *D. suzukii* exploits fruit as a niche. Chemosensory adaptations allow *D. suzukii* to detect chemical cues emitted by ripening and overripe host fruits. Host attraction, consequently, is odour-guided and precedes egg-laying and exploitation of fruit as the larval niche. However, it is not clear to which extent fruit ripeness, presence of yeast, or sex and mating state of the flies, modulate attraction and host choice.

This thesis demonstrates: (i) *D. suzukii* host choice is modulated by fruit ripeness and fly mating, (ii) a reciprocal niche construction and mutualistic interaction between *D. suzukii* and the yeast *Hanseniaspora uvarum* (Niehaus; Ascomycota: Saccharomyceta) on fresh fruit, (iii) the *D. suzukii-H. uvarum* association can be exploited for the development of lures to monitor and control the invasive pest.

The collective findings advance our fundamental understanding of *D. suzukii* host choice decisions and niche construction. This understanding is of relevance for the development of new pest management tools such as manipulation of insect behaviour.

Keywords: fruit-insect-microbe interaction, spotted wing drosophila, yeast, chemical ecology, olfaction, behavioural plasticity, behavioural manipulation, integrated pest management

Señales de frutas y micro-organismos en la ecología del comportamiento y manejo de Drosophila suzukii

Resumen

Investigar los factores que determinan el comportamiento de nuevas plagas es esencial para comprender, predecir y gestionar su impacto en los ecosistemas naturales y agrícolas.

La mosca de alas manchadas (SWD, por su nombre en inglés), *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae), es una plaga polífaga de frutos de piel fina y bayas que se extiende por todo el mundo. *Drosophila suzukii* es capaz de oviponer y desarrollarse en fruta madura, lo que representa un cambio ecológico en relación a las especies de *Drosophila* que prefieren la fruta sobre-madura. Al igual que las *Drosophila* saprófagas, *D. suzukii* vive en estrecha asociación con levaduras, pero la relevancia ecológica de esta asociación es insuficientemente comprendida.

Las señales químicas son importantes cuando *D. suzukii* utiliza la fruta como nicho ecológico. Adaptaciones quimio-sensoriales le permiten a *D. suzukii* detectar las señales químicas emitidas por la fruta en maduración o sobre-madura. En consecuencia, la atracción hacia la fruta hospedera es guiada por el olor, y precede a la puesta de huevos y el consumo de la fruta por parte de las larvas. Sin embargo, aún no está claro cómo la madurez de la fruta, la presencia de levadura, o el sexo y el estado de apareamiento de las moscas, modulan la atracción y la elección del hospedero.

Esta tesis demuestra: (i) que la elección de hospedero en *D. suzukii* está modulada por la madurez de la fruta y el estado de apareamiento de las moscas, (ii) una construcción recíproca de nicho y una interacción mutualista entre *D. suzukii* y la levadura *Hanseniaspora uvarum* (Niehaus; Ascomycota: Saccharomyceta) en fruta fresca, (iii) que la asociación *D. suzukii*-*H. uvarum* puede ser utilizada para el desarrollo de trampas que permitan monitorear y controlar esta plaga invasora.

El conjunto de resultados sobre el comportamiento de *D. suzukii* contribuye a la comprensión sobre las decisiones de *D. suzukii* ante la elección de hospedero y la construcción de su nicho. Este conocimiento es relevante para el desarrollo de nuevas herramientas de manejo de plagas, como ser la manipulación del comportamiento de los insectos.

Palabras clave: interacción fruta-insecto-microorganismo, *Drosophila* de alas manchadas, levadura, ecología química, olfacción, plasticidad del comportamiento, manipulación del comportamiento, manejo integrado de plagas

Dedication

To Franca and Guidaí

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Rehermann G**, González A, Becher PG. Fly mating and fruit ripeness modulates the choice of raspberries by female *Drosophila suzukii*. Manuscript.

II. Chakraborty A, Mori B, **Rehermann G**, Garcia AH, Lemmen-Lechelt J, Hagman A, Khalil S, Håkansson S, Witzgall P, Becher PG. (2022) Yeast and fruit fly mutual niche construction and antagonism against mould. *Functional Ecology*, published online, doi: 10.1111/1365-2435.14054 (in press).

III. Kleman I*, **Rehermann G***, Kwadha CA, Witzgall P, Becher PG. (2022) *Hanseniaspora uvarum* attracts *Drosophila suzukii* (Diptera: Drosophilidae) with high specificity. *Journal of Economic Entomology*, published online, doi.org/10.1093/jee/toac029 (in press).

IV. **Rehermann G**, Spitaler U, Sahle K, Cossu CS, Delle Donne L, Bianchi F, Eisenstecken D, Angeli S, Schmidt S, Becher PG. (2022) Behavioral manipulation of *Drosophila suzukii* for pest control: high attraction to yeast enhances insecticide efficacy when applied on leaves. *Pest Management Science*, 78, 896–904.

V. Spitaler U, Cossu CS, Donne LD, Bianchi F, **Rehermann G**, Eisenstecken D, Castellan I, Duménil C, Angeli S, Robatscher P, Becher PG, Koschier EH, Schmidt S. (2022) Field and greenhouse application of an attract-and-kill formulation based on the yeast *Hanseniaspora uvarum* and the insecticide spinosad to control *Drosophila suzukii* in grapes. *Pest Management Science*, 78, 1287–1295

*Shared first authorship

The contribution of Guillermo Rehermann Del Rio to the papers included in this thesis was as follows:

I. Planned the work with PGB, grew the plants, developed and performed all the experiments, analyzed the data and wrote the manuscript together with PGB and the assistance of AG.

II. Planned the wind-tunnel experiment with PGB and performed the bioassay. Grew and selected unripe and ripe raspberries. Analyzed wind-tunnel, yeast growth and qPCR data. Assisted in writing the manuscript.

III. Planned and developed the work together with co-authors. Assisted in laboratory and field work, analyzed the data and assisted in writing of the manuscript.

IV. Planned and developed the work with US, SS and PGB, performed wind-tunnel bioassays and part of the odor-attraction experiments. Analyzed the data and wrote the manuscript with the assistance of PGB.

V. Assisted planning of the work and data analysis, as well as writing of the manuscript. Paper IV and V are part of the same project.

Publications not contained in this thesis

- Stenberg JA, Sundh I, Becher PG, Björkman C, Dubey M, Egan PA, Friberg H, Gil JF, Jensen DF, Jonsson M, Karlsson M, Khalil S, Ninkovic V, **Rehermann G**, Vetukuri RR, Viketoft M. (2021) When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science*, 94, 665–676, https://doi.org/10.1007/s10340-021-01354-7.

- Kwadha CA, Okwaro LA, Kleman I, **Rehermann G**, Revadi S, Ndlela S, Khamis FM, Nderitu PW, Kasina M, George MK, Kithusi GG, Mohamed SA, Lattorff MG, Becher PG. (2021) Detection of the spotted wing drosophila, *Drosophila suzukii*, in continental sub-Saharan Africa. *Journal of Pest Science*, 94, 251–259, https://doi.org/10.1007/s10340-021-01330-1.

- Mansourian S, Enjin A, Jirle EV, Ramesh V, **Rehermann G**, Becher PG, Pool JE, Stensmyr MC. (2018) Wild African *Drosophila melanogaster* Are Seasonal Specialists on Marula Fruit. *Current Biology*, 28, 3960–3968, https://doi.org/10.1016/j.cub.2018.10.033.

1. Introduction

Chemical language is the most ancient and whispered form of conveying information among living creatures (Haldane, 1955; Wilson, 1970).

Insects are the most diverse group of multicellular organisms on earth, and their success is partly explained by their sensory ability to assess the chemical environment they inhabit. Their sophisticated chemical senses have evolved to detect and discriminate chemical cues in the environment for finding resources (e.g. food, hosts, oviposition sites) and mates, and for avoiding danger (e.g. predators, parasitoids, pathogens) (Hildebrand & Shepherd, 1997; de Bruyne & Warr, 2006). Response to chemical information is important when insects establish their niches and plays a fundamental role in the ecological diversification and speciation of insects (Rundle & Nosil, 2005).

Insect chemical ecology as a discipline studies chemically-mediated interactions between insects and their environments, considering the chemical nature of the information, function of the chemosensory system and behavioural response, as well as the adaptive value of the interaction (Cardé & Haynes, 2004).

Insects are essential elements of the ecosystem, providing services and contributing to ecosystem functions at multi-trophic levels. Insects influence agricultural and horticultural production systems, livestock and human health.

The versatility of insects together with their specialized chemosensory system has led to understanding of fundamental principles in neurophysiology, such us chemical coding and chemosensory circuitry, and how creatures orient and behaviourally respond in a chemo-ecological context. Furthermore, basic and applied studies may help to manipulate and control pest species that threaten food safety and human health (Raguso *et al.*, 2015).

This thesis has focused on an invasive horticultural insect pest, *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae). Combining analytical chemistry and behavioural assays in the laboratory and field, I investigated the ecological relevance of chemical cues meditating interactions between *D. suzukii*, host plants and associated yeasts species. A main goal is to use this knowledge for sustainable pest control.

2. Background

2.1 Insect chemosensation and behaviour

In insects, chemosensory systems are crucial for survival and reproductive success. Chemical information is perceived from conspecifics (e.g. pheromones), from different species (e.g. kairomones) (Brown *et al.*, 1970; Law & Regnier, 1971) as well as from the environment.

Insects are equipped with dedicated peripheral sensory systems for detecting chemical information, which are tuned to compounds of ecological relevance rather than to broadly sample the chemical environment (Bohbot & Pitts, 2015). Peripheral systems are connected to brain areas where the chemical information is processed, integrated and transduced into physiological response, such as behaviour.

Much of our current understanding of chemical communication, physiology and behaviour is based on research on the model organism *Drosophila melanogaster*, closely related to *D. suzukii*. Comparative studies of the ecology and behaviour of *Drosophila* flies can provide basic understanding of chemical and behavioural ecology, and moreover be of practical use.

In the following brief overview on insect chemosensation and behaviour I will focus on *Drosophila* flies.

2.1.1 Chemosensory organs and receptors: the sensory input

Chemosensation can be divided into the senses of smell (olfaction) and taste (gustation) based on the spatial scale. While the former can be defined as distant-chemoreception (airborne odorants), gustation could be considered as contact-chemoreception, which in general requires contact with chemicals supplied on substrates (de Bruyne & Warr, 2006).

In *Drosophila* flies, as in most other insects, odorants are primarily detected by the antennae, as the insect "nose". In addition, flies are equipped with a pair of maxillary palps, forming together with the antennae, the olfactory organs of adult flies. Both appendages are covered with specialized porous cuticular hairs called sensilla, that house olfactory sensory neurons (OSNs), which express in the dendritic membrane distinct olfactory chemoreceptors: odorant receptors (ORs), ionotropic receptors (IRs) or some of the gustatory receptors (GRs) (Clyne *et al.*, 1999; Vosshall *et al.*, 1999; Benton, 2015). OSNs are bipolar, and their axons project directly to the brain.

Taste detection, on the other hand, involves gustatory sensilla distributed over multiple appendages of the fly body surface, including antennae, mouthparts, legs, wings, genitalia and other parts of the abdomen (Stocker, 1994, Depertis-Chauvin *et al.*, 2015). Axons of gustatory receptor neurons (GRNs) do not converge to a brain area directly, but rather converge in their appendages, connecting to the central nervous system (Stocker, 1994).

Larval olfactory and gustatory organs differ substantially in anatomy and number of OSNs from their adult counterpart (Voshall & Stocker, 2007). This is not surprising considering that larvae and adults are distinct in their lifestyle and niches, and undergo extensive anatomic changes during their holometabolous development. In larvae, the main chemosensory structures are localized in the head. While larval ORNs are confined to the dorsal organs, GRNs are localized in the terminal, ventral, and in reduced numbers in the dorsal organs as well as in several pharyngeal organs (Voshall & Stocker, 2007; Depetris-Chauvin *et al.*, 2015).

2.1.2 Chemosensory pathway: where the stimuli are processed and integrated

The number of odorants in the world is inestimable, but only few are of relevance for the receiver and detected by chemosensory receptors.

In brief, OSNs expressing the same OR transmit information via axonal projections to the same specific glomerulus in the antennal lobe of the brain. The glomeruli receive information from OSNs and local interneurons that connect different glomeruli. As a result, the glomeruli are activated in a pattern that is decoded in higher brain centres for learning, memory and behaviour (Zars, 2000; Masse et al., 2009).

Overall, to date, remarkable progress has been made in understanding the neural circuity and mechanisms underlying the detection of info-chemicals (e.g. Vosshall & Stocker, 2007; Joseph & Carlson, 2015 and references there in). However, investigations on how particular sensory input relates to a behavioural output and the underlying genetic mechanisms remains to be understood in more detail.

2.1.3 Chemosensory-mediated behaviour: the behavioural output

Behaviour interfaces the sensorial input of an organism with its ecological interactions. Behavioural responses to chemical cues that signal the availability of food, mating partners, or oviposition sites are essential for survival and reproductive success and thus, substrate for selection.

Generally spoken, ecologically relevant chemical information perceived by an organism can trigger behavioural attraction (e.g. food, breeding sites, conspecific), aversion (e.g. toxin, predator), or activate more complex behavioural programs such as courtship, egg-laying or aggression.

In *Drosophila* species, adaptations to host plants is to a large extent expressed in behaviours that parallel olfactory shifts. Such adaptations get visible in the distinct host selection of different species such as *D. melanogaster* which uses various kinds of overripe fruit, *D. suzukii* which exploits a high diversity of soft-skinned fruit at different maturation stages, or *Drosophila sechellia* and *Drosophila erecta,* which are specialists on *Morinda citrifolia* or *Pandanus* sp. fruits, respectively (e.g. Dekker *et al.*, 2006; Linz *et al.*, 2013; Karagerogi *et al.*, 2017; Mansourian *et al.*, 2018).

Microbes, on the other hand, occupy a trophic level that links insects with their host plants. Most *Drosophila* species, if not all, are intimately associated with yeast species (Phaff *et al.*, 1956; Begon, 1982). Yeasts are found in fly habitats and substrates such as leaves or fruit, as well as on and inside the *Drosophila* body (Chandler *et al.*, 2012; Douglas *et al.*, 2015). Yeast volatiles may signal to flies suitable feeding or oviposition sites (Becher *et al.*, 2012). Thus, behavioural responses to yeast metabolites might be of relevance for *Drosophila* ecology and niche occupation (Buser *et al.*, 2014; Koerte *et al.*, 2020).

2.1.4 Chemosensory and behavioural plasticity

Insects can adjust their behaviour in response to a changing chemical environment. Likewise, peripheral detection and central nervous processing of odorants may be modulated for example in response to the feeding or mating state, or experience of the insect. Thus, plasticity in odour guided behaviours allows insects to respond to odour cues in an adaptive way (Anton & Rössler, 2021 and references therein). As an example, *D. melanogaster* response to food odour cues is modulated by hunger. A stronger attraction to food chemical cues was observed on starved flies relative to fed individuals (Edgecomb *et al.*, 1994; Becher *et al.*, 2010). Behavioural response to sexual cues can also be modulated on a mating-dependent manner. For example, mating suppresses the attraction towards a male sex-pheromone in *D. melanogaster* females (Lebreton *et al.*, 2015).

2.1.5 Assessing chemosensory-mediated behaviour

Designing a sensitive and discriminative bioassay is crucial to answer research questions about behaviour. Some laboratory bioassays are rather "simple" but still allow robust qualitative and quantitative measurements of chemosensory-mediated behavioural response in flies. However, behavioural assays benefit from detailed knowledge of the biology, physiology, and ecology of the tested individuals.

Any behavioural assay is limited in explanatory power and assessment of multimodal behaviour may require the combination of complementing assays to avoid misinterpretation of behavioural data. Moreover, assays often measure the end result of a behavioural output (e.g. number of eggs laid after 1 d) disregarding the temporal sequence of behavioural decisions (e.g. which oviposition substrate was chosen first).

Courtship, mating and oviposition assays allowed the development of behavioural paradigms in *Drosophila* flies, but our understanding of most basic behaviours is still incomplete (e.g. Becher *et al.*, 2012; Stensmyr *et al.*, 2012; Dweck *et al.*, 2013, 2020; Billeter & Levine, 2015; Auer & Benton, 2016; Bräker *et al.*, 2019; Khallaf *et al.*, 2020). Tracing and explaining subtle behaviours that interconnect multi-sensory evaluation remains a challenge. For example, odour-guided attraction and preference can be re-evaluated at short range by gustatory inputs resulting in a mismatch between the initial attraction and the final oviposition site preference (Karageorgi *et al.*, 2017;

Koerte *et al.*, 2020). Thus, there is a need to identify faint elements, interconnection, and temporal coding of behavioural responses.

While observation and recording allow measuring of behaviour, sensory stimulation is one of the factors that allow controlled experimental manipulation. For odour-guided behaviours, odorant identity, compound concentrations and ratios of multicomponent mixtures constitute relevant information to the organism and may affect behavioural response (Visser *et al.*, 1986; Stensmyr *et al.*, 2003; Tasin *et al.*, 2006).

Behaviour can be a response to complicated stimulus interactions such as synergistic or antagonistic stimulation by individual odour components (Becher *et al.*, 2010). Moreover, odours of distinct origin, such as plants, microbes or insects, intermix and interact (Grosjean *et al.*, 2011; Lebreton *et al.*, 2017). Systematic testing of odorants as single compounds or in mixture allows to detangle the activity of complex odour blends such as samples of plant or microbial headspace.

In this PhD study, well-established and new experimental designs and protocols were used to examine *D. suzukii* behavioural responses to chemical information (see *Main research approach and methodology* section and *Papers* for further details).

2.2 Chemical ecology and behavioural manipulation for pest management

Chemical information drives relevant interactions at different ecological levels in natural and human-managed ecosystems. Understanding of the mechanisms underlying insect chemosensation may allow to predict chemosensory-guided behaviour and to manipulate the behaviour of economically important insects such as pollinators, disease vectors, and other pests (Raguso *et al.*, 2015; Reisenman *et al.*, 2016).

Aware of the broad scope of the topic, in this section I focus on agricultural and horticultural pest systems which is of most relevance for this thesis (for a comprehensive review of pollinators or harmful disease vectors readers are advised to consult e.g. Raguso, 2008; Reisenman *et al.*, 2016; Maia & Moore, 2011).

The aim of behavioural manipulation of insect pests by info-chemicals is to stimulate or inhibit a behaviour, or modify its expression, in order to negatively impact pest performance and life cycle, and consequently to reduce crop damage (Wright, 1964; Foster & Harris, 1997; Allan, 2018).

Behavioural manipulation tactics are based on the use of signals or cues that mediate intraspecific as well as interspecific interactions. A well-known example that exploits insect communication is the use of sex pheromones (Witzgall *et al.*, 2010 and references therein). The high reliance on sex pheromones for mate location in some insect species, makes the use of pheromones an important tool within pest management strategies (Witzgall *et al.*, 2010). Mating-disruption, as a well-established management tool in horticultural systems is based in the release of synthetic pheromones in amounts that permeate the chemical information landscape to hinder mate-finding and thus prevent mating (Witzgall *et al.*, 2008; Miller & Gut, 2015). In addition, aggregation and alarm pheromones (from conspecifics or heterospecifics) have been used to manipulate olfactory behaviour to attract or repel insects in the field (Cook *et al.*, 2007 and references there in). The "push and pull" strategy combines repellents and attractants in the same environment (Cook *et al.*, 2007). Pheromones are moreover efficiently used as attractant lures for species detection and population monitoring, both for established pests as well as invasive species (Witzgall *et al.*, 2010). Traps baited with pheromones can also be used for mass trapping or to "attract-and-kill" pests (Witzgall *et al.*, 2010; Heuskin *et al.*, 2011). Mass trapping and attract-and-kill are similar tactics, but differ in the method of killing the insect after attraction. While the former typically kills the insect by a drowning solution or contact adhesive, attract-and-kill typically applies a toxicant (El-Sayed *et al.*, 2006, 2009). Attract-and-kill can also be applied using semio-chemicals different to pheromones (see below).

Application of non-pheromone info-chemicals is a less widespread practice, but behavioural manipulation of insects by (non-)host or microbial chemical cues has been demonstrated (e.g. Cook et al., 2007; Rodriguez-Saona *et al.*, 2009; Davis *et al.*, 2013; Knight & Witzgall, 2013). For example, host and floral volatiles can be used in attractant bait traps (Del Socorro *et al.*, 2010; Gregg *et al.*, 2010). Conversely, plant volatiles emitted by herbivory-induce attack can repel targeted organisms and attract natural enemies (Turlings *et al.*, 1995). Synthetic plant volatiles or plants that constitutively emit these volatiles can be used for host avoidance and biological control enhancement (Cook *et al.*, 2007). Combination of pheromone and habitat cues is an on-going research area (e.g. Hatano *et al.*, 2015; Borrero-Echeverry *et al.*, 2018; Gonzalez *et al.*, 2020). On the other hand, essential oils from non-host plants can be used to elicit repellence (Wang *et al.*, 2021).

Microbial volatiles can attract insects, mediate food, host or mate finding, as well as trigger aversion or avoidance behaviours (e.g. Becher *et al.*, 2010, 2012, Stensmyr *et al.*, 2012). Distinct yeast species emit species-specific volatile signatures to which insects respond discriminative (Scheidler *et al.*, 2015; Ljunggren *et al.*, 2019; Koerte *et al.*, 2020). Understanding specific insect yeast symbioses may lead to the development of pest management tactics (Douglas, 2007). Similar to the tactics implemented using sex pheromones, yeast and synthetic yeast volatiles have been suggested as attractant lures for early detection and population dynamics as well as for attract-and-kill formulations (Witzgall *et al.*, 2012; Hamby & Becher, 2016; Bueno *et al.*, 2020). Yeast can be used in combination with insecticides and attract pests to toxic baits and stimulate ingestion (Knight *et al.*, 2016; Hamby & Becher, 2016; Mori *et al.*, 2017). In addition, yeast can be used for attract-and-infest tactics, where the pest is odour-attracted to a devise containing yeast and a pathogenic inoculum. In difference to attract-and-kill, infested individuals can leave the devise to disseminate infection among conspecifics (Shah & Pell, 2003; Yousef *et al.*, 2018).

2.3 *Drosophila suzukii*

Drosophila suzukii (Matsumura; Diptera: Drosophilidae) is one of the approximately 1,500 known species of the genus *Drosophila* commonly referred to as vinegar flies (Markow & O'Grady, 2006). Native to Southeast Asia (Kanzawa, 1939), over the past decade *D. suzukii* has gained attention of fruit growers and industry, as well as the scientific community.

Why? Because *D. suzukii* has emerged as a severe invasive pest on soft-skinned fruit such as cherries, raspberries, blueberries and strawberries (Hauser *et al.*, 2011; Lee *et al.*, 2011; Walsh *et al.*, 2011).

How? Evolutionary explanation is given by occurrence of specific adaptations, such as a prominent serrated ovipositor that enables female *D. suzukii* to pierce the fruit skin (Atallah *et al.*, 2014), and sensory physiological characteristics underlying behavioural response towards ripening fruit (e.g. Keesey *et al.*, 2015; Ramasamy *et al.*, 2016; Karageorgi *et al.*, 2017; Durkin *et al.*, 2021; Dweck *et al.*, 2021). Remarkably, the *D.*

suzukii-specific changes are accompanied by a number of traits that are shared among many *Drosophila* and most likely contributed to *D. suzukii´s* fitness. For instance, high reproductive output, short generation time, a wide range of feeding and oviposition sites, as well as a close association with naturally occurring yeasts, other fungi, and bacteria (Hamby & Becher, 2016; Little *et al.*, 2020; Tait et al., 2021). As a result, *D. suzukii* is highly adaptable to new environments, which together with the international trading of commodities, has facilitated *D. suzukii* to spread widely and rapidly (Asplen *et al.*, 2015; dos Santos *et al.*, 2017; Kwadha *et al.*, 2021).

Thus, since its global spread to fruit producing regions, *D. suzukii* has been extensively studied as comparative model for *Drosophila* ecology and evolution, as well as a target for pest control strategies (e.g. Karageorgi *et al.*, 2017; Dweck *et al.*, 2021; Tait *et al.*, 2021) (Figure 1).

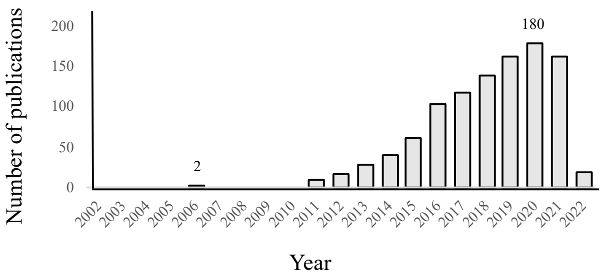

Figure 1. Record of publications on *Drosophila suzukii* over the last 20 years. Data obtained from Web of Science Core Collection (https://www.webofscience.com, accessed on 2022.04.10). Search: ("*Drosophila suzukii*" OR "Spotted wing drosophila"). Total number of publications: 1.051.

Drosophila suzukii is highly polyphagous, with at least 198 plants species representing 73 genera getting infested by the fly (Little *et al.*, 2020). Moreover, several maturation stages of fruit as well as wounded fruit can be exploited by *D. suzukii* (Lee *et al.*, 2011; Arnó *et al.*, 2016; Kienzle *et al.*, 2020).

In brief, the exploitation of fresh fruit by *D. suzukii* is intimately linked to the evolution of a distinctive sclerotized and serrated ovipositor to penetrate the skin of ripening fruits; females insert their eggs and larvae feed and develop inside the pulp (Atallah *et al.*, 2014; Green *et al.*, 2019). Host selection behaviour came along with several chemo and mechano-sensory adaptations related to *D. suzukii* egg-laying (Karageorgi *et al.*, 2017; Dweck *et al.*, 2021; Crava *et al.*, 2020). *Drosophila suzukii* tolerates harder substrates for egg-laying than other *Drosophila* species (Karageorgi *et al.*, 2017; Durkin *et al.*, 2021), which is congruent with the higher skin firmness of ripening fruit compared to overripe. In addition, while some drosophilids, including *D. melanogaster*, avoid oviposition in bitter-enriched substrates typically present in immature fruit, significant loss of sensing bitterness by *D. suzukii* supports an important role of gustation in the preference of fruit (Dweck *et al.*, 2021). What is more, ripe strawberry fruit odours are sufficient to elicit oviposition behaviour in *D. suzukii* females (Karageorgi *et al.*, 2017). Thus, *D. suzukii* oviposition decisions are guided by stimuli targeting different sensory modalities. Despite much effort, we are still at the beginning to unravel *D. suzukii* physiology and behaviour. Modulation of the odour-mediated preference for ripening fruit, and egg-laying behaviour were studied in Paper I.

Fruit is habitat for *D. suzukii* as well as microorganisms. Yeasts are intimately linked to the ecology of *D. suzukii* similar as known for other *Drosophila* species. For instance, yeasts are found inside and outside fruit and *D. suzukii* flies (Hamby *et al.*, 2012). Indeed, yeasts are sufficient to support *D. suzukii* larval development and adult survival (Belluti *et al.*, 2018; Spitaler *et al.*, 2020), and yeast volatiles induce *D. suzukii* behavioural responses (Scheidler *et al.*, 2015).

Hanseniaspora uvarum (Niehaus; Ascomycota: Saccharomyceta) is an apiculate yeast wildly distributed in fruit plantations (Bokulich *et al.*, 2014). Particularly, *H. uvarum* is known in the beverage industry for its aromatic capabilities (González-Robles *et al.*, 2015; Mestre *et al.*, 2019), as well as for biocontrol of fruit pathogens (Liu *et al.*, 2010; Qin *et al.*, 2017; Koutsoumanis *et al.*, 2019). Remarkably, *H. uvarum* is the predominant yeast associated with *D. suzukii* (Chandler *et al.*, 2012; Hamby *et al.*, 2012). *Drosophila suzukii* shows a strong olfactory response and high attraction towards *H. uvarum* volatiles (Scheidler *et al.*, 2015; Mori *et al.*, 2017). Moreover, when offered as food *H. uvarum* increased the *D. suzukii* reproductive output more than other yeasts that were found in association with the fly (Spitaler *et al.*, 2020). Altogether this has placed *H. uvarum* into the focus of *D. suzukii*-yeast research aiming at fundamental understanding of the ecological association. Paper II investigated *D. suzukii*–*H. uvarum* interactions in the context of the fruit, as its shared habitat. Moreover, *H.*

uvarum is considered as a promising candidate for the development of *D. suzukii* management strategies.

In the past decade, substantial research effort has been made to develop and optimize trap design and lures to attract, attract-and-kill, or repel *D. suzukii* as complementary methods to reduce pesticide application for *D. suzukii* management (Tait *et al.*, 2021 and references therein). Not surprisingly, lures based on ripe or fermenting fruit scents, apple cider vinegar alone or combined with wine as well as commercial food baits have shown variable efficacy in trapping target and unwanted non-target species (Cloonan *et al.*, 2018; Tait *et al.*, 2021). The natural association between *D. suzukii* and *H. uvarum* (or other co-occurring microbes) provides an ecological foundation to pave an avenue for the development of highly specific management strategies integrating measures of pest prevention and control. Papers III-V of this thesis investigated the implementation of *H. uvarum* in behaviour-modifying tactics for *D. suzukii* management. While paper III studied the attraction of the flies to yeast volatiles in the field, papers IV and V explored the application of the yeast in an attract-and-kill technique as a proof of concept for novel pest control.

3. Aims and Hypotheses

The main aim of this thesis was to investigate the relevance of fruit and microbial cues in the behavioural ecology of *Drosophila suzukii*.

The focus was on the interplay between fruit-fly and fly-yeast interactions in *D. suzukii* decision-making, with the aim to provide scientific background for development of efficient pest control methods.

The first part of this thesis (Paper I) investigates how fruit ripening influences *D. suzukii* host preference, mating, and egg-laying behaviour.

The main hypothesis was:

i) Odour-mediated D. suzukii behaviour is modulated in response to fruit ripeness and fly mating.

The second part of this thesis (Paper II) provides a closer look at the ecological significance and implications of fly-yeast interactions with focus on *Hanseniaspora uvarum*, a yeast closely associated with *D. suzukii*

The main hypothesis was:

i) The interaction with H. uvarum facilitates D. suzukii´s exploitation of fruit.

The third and final part of this thesis (Papers III – V) describes studies on the attraction of *D. suzukii* to *H. uvarum* volatiles for manipulating *D. suzukii* behaviour in pest management.

The main hypotheses were:

i) Hanseniaspora uvarum volatiles attract D. suzukii with high specificity (Paper III).

ii) Hanseniaspora uvarum in combination with insecticides induces D. suzukii attraction and contact, which improves insecticide efficacy (Paper IV).

iii) Hanseniaspora uvarum-insecticide formulations applied on canopy reduces D. suzukii fruit infestation and pesticide residues (Paper V).

4. Main research approach and methodology

4.1 Scope

The main experimental approach of this thesis was to control sensory input (chemosensory stimuli) and to quantify behavioural output of *D. suzukii*. The study focus was on odour blends of ecological relevance that modulate *D. suzukii* behaviour. Moreover, the study had an applied perspective with respect to behavioural manipulation for pest management. Sensory processing was not in the scope of this study.

4.2 Organisms

Flies

The *D. suzukii* fly stocks for behavioural assays at the Swedish University of Agricultural Sciences (SLU), Alnarp, originated from infested fruit collected in Northern Italy (South Tyrol or San Michele all'Adige, Italy). Flies were maintained at 22–24°C and 35–65% R.H., under a 12:12 h L:D (light:dark) photoperiod on a sugar-yeast-cornmeal diet.

Plants and fruits

Plant and fruit materials varies along and among the different experiments. As an example, raspberries (*Rubus idaeus L.*) were obtained from plants grown at one of SLU Alnarp´s greenhouses, from a local farm (Hallongården, Trelleborg, Sweden), or from grocery shops. Similarly, *Vitis vinifera L.* leaves and grapes used for behavioural assays were locally grown at the SLU Alnarp´s vineyard, or from Laimburg Research Center, Ora, Italy, or obtained from local producers and markets.

Microorganisms

Hanseniaspora uvarum strains were two. *Hanseniaspora uvarum* - CBS 2570 was obtained from the Centraalbureau voor Schimmelcultures (Utrecht, Netherlands) (Paper II and III), and *H. uvarum* -LB-NB-2.2 was isolated from feeding grooves of *D. suzukii*-infested grapes in South Tyrol, Italy (Paper IV and V).

4.3 Behavioural assays

4.3.1 Larval behaviour

Larval responses to chemical cues can be measured on static agarose Petri dish assays where stimuli are loaded on filter paper. Various studies have used a comparable bioassay demonstrating attraction of *Drosophila* larvae towards single fruit or yeast odours as well as to odours blends (e.g. Fishilevich et al., 2005; Dweck et al., 2018).

Paper II of this thesis studied *D. suzukii* larval attraction and aversion towards microbial chemical cues such as three species of yeast and mould in the context of host choice.

4.3.2 Flight behaviour

To examine adult attraction to food or host airborne chemical stimuli, a wind tunnel is a powerful bioassay. Up-wind oriented flight behaviour is conspicuous and unambiguous. It can be measured and represents long-range odour-guided attraction towards volatiles cues (Budick & Dickinson, 2006; Becher et al., 2010). In short, individuals are released at the down-wind end of the tunnel and exposed to a main air stream carrying a plume of stimulus odour. Wind tunnel experiments have shown versatility with regards to the tested stimuli per-se (e.g. plant, fruit, microorganisms, insects or combinations; single chemical stimuli or synthetic mixtures) and the odour-delivery method (e.g. headspace emissions in vivo, piezoelectric sprayer of chemicals at a known constant rate and purity).

Examining odour guided up-wind flight behaviour of *D. suzukii* was a central task in the current study (Papers II-IV) (Figure 2).

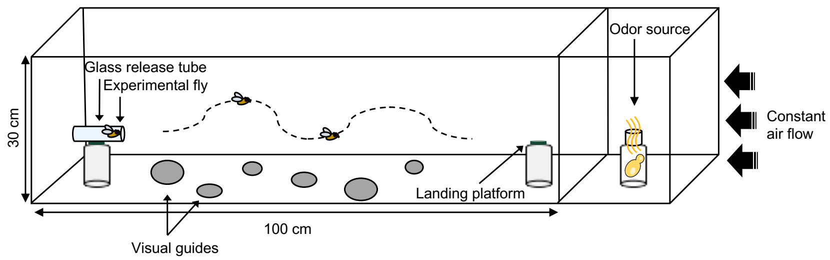

Figure 2 . Scheme of the flight wind tunnel setup

4.3.3 Odour-evoked behavioural preference

In an olfactometer, individuals are exposed to an air stream containing specific odorants or combinations, ideally delivered in precisely controlled manner. Olfactometers offer the possibility to evaluate behaviour and preference towards odour stimuli in a multi-choice arrangement (e.g. Biasazin et al., 2019).

Accordingly, a six-choice olfactometer (Biasazin et al., 2019) was employed in this study to determine the odour-driven preference of *D. suzukii* for different ripening stages of fruit (Paper I) (Figure 3).

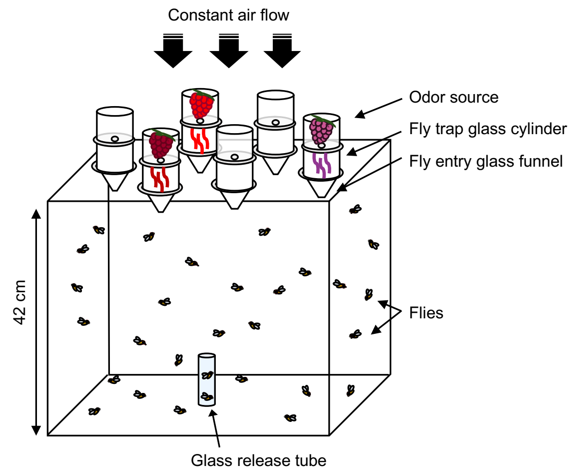

Figure 3. Scheme of the six-choice olfactometer setup.

4.3.4 Oviposition behaviour

Oviposition decision-making involves the exploration of suitable breeding sites prior and during egg-laying by multiple sensory channels in a coordinated fashion (Bräker et al., 2019), but the role of chemosensation is crucial (e.g. Stensmyr et al., 2012; Becher et al. 2012; Karageorgi et al., 2017; Mansourian et al., 2018; Dweck et al., 2021).

In Paper I and II we investigated *D. suzukii* egg-laying behaviour with respect to fruit and microbial cues to examine oviposition preference or avoidance. In paper IV and V egg-laying was assessed not as direct result of chemosensory mediated behaviour, but to measure the effect of insecticide treatment.

For detailed information readers are referred to Materials and Methods of the corresponding papers.

5. Main results

5.1 Part I: Fruit and flies – Fruit ripening and *Drosophila suzukii* mating state modulate host choice

Raspberry fruit (*Rubus idaeus* L. 'Glen Ample') is one of the most preferred and highly susceptive hosts for *D. suzukii*. Ripening fruits were categorized in four progressing ripening stages: unripe, semi-ripe, ripe and overripe, characterised by distinct coloration, volatile profiles and skin firmness (Figure 4, Paper I).

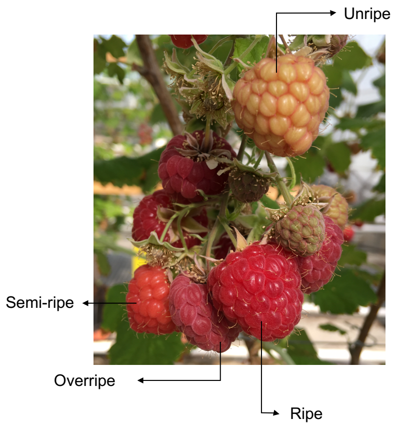

Figure 4. ***Rubus idaeus L. 'Glen Ample' fruit ripening stages***

Mating induces great physiological changes in most insects and can modulate behavioural decision-making in response to sensory stimuli. Under the hypothesis that *D. suzukii* mating induces distinctive behaviour towards host cues, we first examined the odour-mediated behaviour of *D. suzukii* in response to scents emitted by semi-ripe, ripe or overripe raspberries. We demonstrated that virgin *D. suzukii* females were similarly attracted to ripe and overripe fruit, while mated females clearly preferred ripe fruit. Semi-ripe berries were less preferred (Figure 5).

Our results showed that mating modulates odour-guided preference for ripening raspberry fruits in *D. suzukii* females.

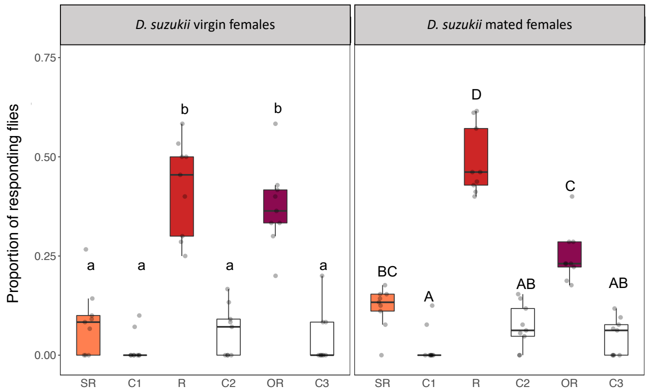

Figure 5. Odour-mediated attraction of *Drosophila suzukii* towards raspberries of different ripening stage. Preference (after 60 min) of groups of 30 *Drosophila suzukii* virgin (left), and mated (right) females when exposed in an olfactometer to odours of semi-ripe (SR), ripe (R) and overripe (OR) raspberries *Rubus idaeus L.* Fruit-odours were offered simultaneously with blank controls (C1, C2, C3) placed in between fruit stimuli. The boxes represent the interquartile range divided by the median, and whiskers represent the data within 1.5× the interquartile range. Dots represent the data distribution of the individual replicates. Different letters above boxes describe significant difference after multiple comparisons of means ($P < 0.05$; lower case for *D. suzukii* virgin females, uppercase for *D. suzukii* mated females). Figure modified from Paper I.

With the aim to unravel the ecological relevance of fruit cues in the context of reproductive behaviour, we speculated that the preference of mating onto fruit of different ripening stages correlates with the fruit odours most attractive to yet unmated females i.e. the preference for ripe and overripe raspberries. When offered green raspberry leaves, semi-ripe, ripe and overripe raspberries in a mating assay, we first determined that courtship and mating do not take place at random sites, but on the fruit. In addition, mating occurred at similar rates in ripe and overripe fruit which were the preferred mating sites followed by semi-ripe raspberries, leaf shoots or any other space inside the test cage (Figure 6).

These results show that fruit cues might indicate a suitable *rendez-vous* and mating site for *D. suzukii*. More than that, the data suggest a correlation between odour-guided preference and mating site preference in virgin *D. suzukii* flies (Paper I).

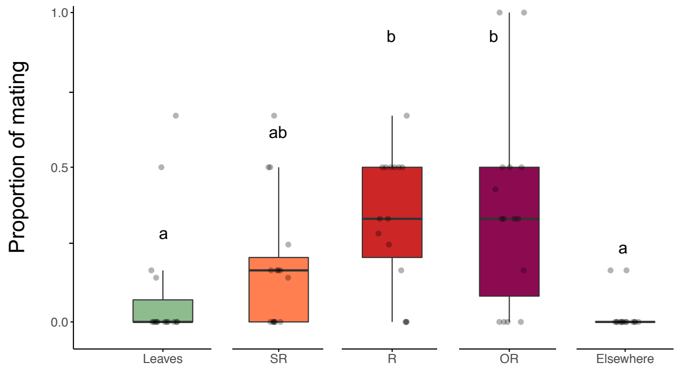

Figure 6. *Drosophila suzukii* mating site preference. Mating site preference (after 60 min) of groups of ten *D. suzukii* virgin females and males when exposed to of semi-ripe (SR), ripe (R) and overripe (OR) raspberries *Rubus idaeus* L. and a raspberry leaf shoot. The boxes represent the interquartile range divided by the median, and whiskers represent the data within 1.5× the interquartile range. Dots represent the data distribution of the individual replicates. Different letters above boxes describe significant difference after multiple comparisons of means ($P < 0.05$). Figure from Paper I.

We then investigated whether fruit ripening modulates *D. suzukii* female oviposition choice. We hypostatized that the choice for egg-laying correlates with the odour-mediated preference of mated females for ripe fruit. Our results showed that most of the females preferred landing on ripe fruit, followed by landings on overripe, semi-ripe and unripe, respectively (Figure 7A), which is congruent with the odour-mediated preference for ripe fruit previously demonstrated. Moreover, we showed that despite of the ability to discriminate raspberries of different ripeness by olfaction, *D. suzukii* females exploited all the ripening stages for egg-laying. Remarkably, however, females laid most eggs on ripe fruit and insignificantly less on overripe fruit, followed by the two more unmatured stages that were less preferred (Figure 7B). Thus, odour cues mediate both landing and oviposition in *D. suzukii*.

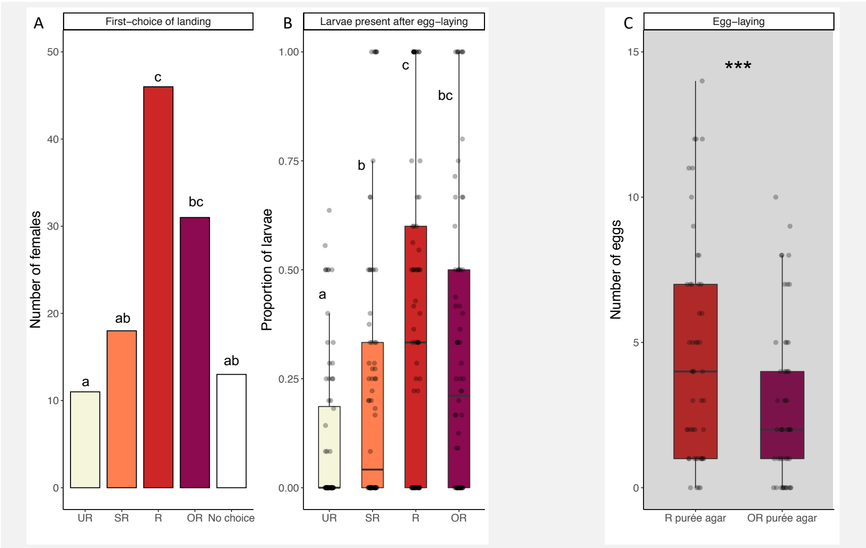

Figure 7. *Drosophila suzukii* oviposition decision-making on raspberries of different ripeness. A) First-choice of landing of mated *D. suzukii* females exposed individually to four ripening stages of raspberries (*Rubus idaeus L.*) simultaneously: unripe (UR), semi-ripe (SR), ripe (R) and overripe (OR), and B) egg-laying, quantified from the number of larvae inside the fruit. C) Oviposition preference measured as number of eggs laid by *D. suzukii* females when exposed individually to two different oviposition substrates: R and OR raspberry purée of equal firmness tested in darkness. Dots represent the data distribution of the individual replicates. Different letters above boxes illustrate significant difference in response to the ripening of the fruit after multiple comparisons of means (P < 0.05). Asterisks denote significant differences in the number of eggs between fruit purées substrates (*** P < 0.001). Figure modified from Paper I.

Fruit skin firmness decreases with progressing raspberry maturation (Paper I). To unravel the interplay between fruit firmness (mechano-sensation) and chemical composition (chemo-sensation) in oviposition decisions, we performed a two-choice assay exposing individual females to substrates made of raspberry purées that were of equal firmness but differed in their chemo stimulatory composition as prepared from ripe or overripe berries. Notably, we demonstrated a strong oviposition preference for purée made of ripe compared to overripe fruit (Figure 7C).

Overall, these results highlight a *D. suzukii* behavioural plasticity in host seeking and oviposition behaviour, which is modulated by mating and fruit ripeness. These findings advance our understanding of *D. suzukii* odour-mediated behaviours. Furthermore, this and other recent studies, provide evidence that odour information is of relevance, but not the sole stimulation that determines oviposition choice, suggesting a complex interplay between sensory modalities in oviposition decision-making (Karageorgi et al., 2017; Durkin et al., 2021; Dweck et al., 2021).

5.2 Part II: Fruit, flies and fungi – *Drosophila suzukii* and *Hanseniaspora uvarum* association on fruit leads to reciprocal niche construction

Microbes link insects with their host plants. We studied *D. suzukii* - *H. uvarum* interactions and reciprocal benefits in the context of a shared fruit habitat. We hypothesized that a mutualistic relation between *D. suzukii* and *H. uvarum* facilities the exploitation of fruit for both organisms.

We first investigated whether *D. suzukii* adults and larvae were attracted to volatiles emitted by yeast and whether they were able to discriminate yeast species based on their characteristic volatile signature. In adult flight attraction (Figure 8) and oviposition assays, as well as larval attraction assays, *D. suzukii* responded stronger to *H. uvarum* volatile metabolites compared to *Saccharomyces cerevisiae* and *Pichia terricola* yeasts. Thus, *D. suzukii* can discriminate yeast identity based on species-specific volatile signatures and preferred those emitted by *H. uvarum*. Moreover, *H. uvarum* supported higher larval development and adult survival than S. *cerevisiae*. In return, we observed that *H. uvarum* growth and dispersal is promoted by *D. suzukii* (Paper II).

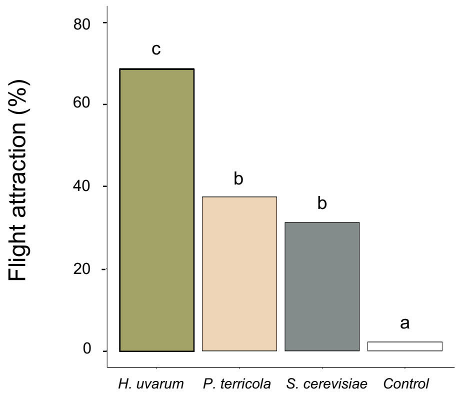

Figure 8. Flight tunnel attraction of *Drosophila suzukii* to yeast volatiles. Up-wind flight behaviour (%) of mated *D. suzukii* females towards headspace volatiles of *Hanseniaspora uvarum*, *Pichia terricola*, *Saccharomyces cerevisiae*, and control (growth medium without yeast). Different letters denote significant difference in yeast attractiveness ($P < 0.05$). Figure modified from Paper II.

We further investigated if *D. suzukii*–*H. uvarum* interactions shape their shared fruit habitat in antagonism to detrimental grey mould, *Botrytis cinerea*. We found out that *H. uvarum* and *D. suzukii* suppressed the growth of the antagonistic *B. cinerea* on raspberry fruit (Figure 9).

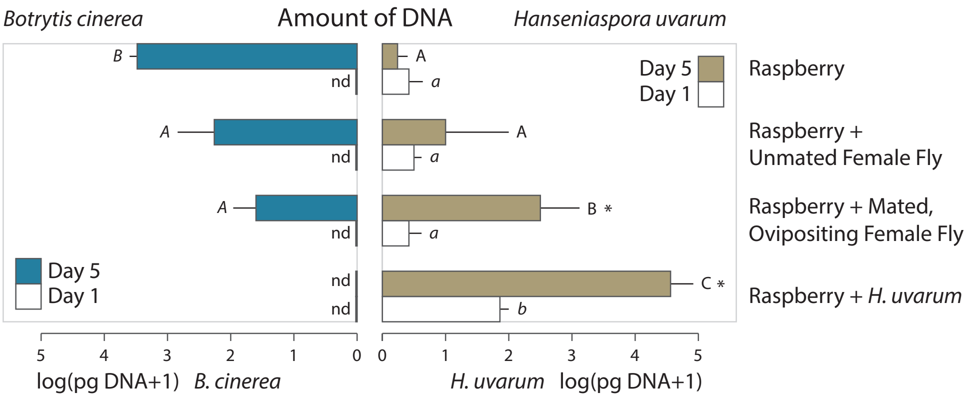

Figure 9. Effect of *Drosophila suzukii* on *Hanseniaspora uvarum* yeast and *Botrytis cinerea* mould development in *Rubus idaeus L.* raspberries. Amount of DNA (Mean ± SD) of *H. uvarum* and *B. cinerea* extracted after 1 and 5 d from individual untreated, field-collected raspberries (control), raspberries exposed to unmated or to mated, ovipositing *D. suzukii* females, or raspberries dipped into a culture of *H. uvarum*. Different letters show significant differences in DNA amounts, between treatments, after 1 d (small letters) and 5 d (capital letters). Asterisks show differences in DNA amounts, for the same treatment, on different days. nd means no-detected. Figure from Paper II.

These results show that the interaction between *D. suzukii* and *H. uvarum* is mutualistic. Co-occurring on fruit, fly and yeast engage in niche construction in a reciprocal manner. This is in line with the close association of *D. suzukii* and *H. uvarum* observed in nature (Hamby et al., 2012; Hamby & Becher, 2016).

5.3 Part III: A fly associated yeast for pest management – implementation of *Hanseniaspora uvarum* for behavioural manipulation of *Drosophila suzukii*

Insects can be manipulated with volatile metabolites that are relevant in their behavioural ecology. The natural association and mutualistic interaction between *D. suzukii* and *H. uvarum* described in Part II motivated us to advance our studies on the attraction of *D. suzukii* to *H. uvarum* for manipulating *D. suzukii* behaviour, as contribution to pest management.

Extracts of *H. uvarum* headspace volatiles induced *D. suzukii* flight attraction. Furthermore, a synthetic blend of few specific *H. uvarum* volatiles was sufficient to trigger strong up-wind attraction (Figure 10A). When tested in the field, traps baited with *H. uvarum* headspace extracts and a drowning solution, showed specificity for trapping *D. suzukii*, comparable or even higher to a commercial bait or synthetic references lures, that were based on wine and vinegar components (Figure 10B, Paper III).

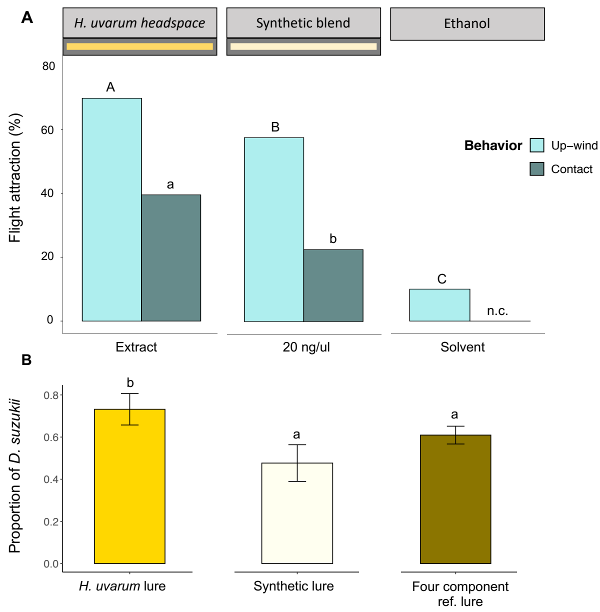

Figure 10. **Flight tunnel attraction and field trapping of *Drosophila suzukii*.** A) Up-wind flight behaviour and contact (%) with the odour source of virgin *D. suzukii* females towards vaporized *Hanseniaspora uvarum* headspace extract, a synthetic blend of seven *H. uvarum* volatiles, and to ethanol. B) Proportion (Mean ± SEM) of *D. suzukii* relative to other trapped arthropods captured with lures based on *H. uvarum* headspace (*H. uvarum* lure), a synthetic blend of *H. uvarum* volatiles (Synthetic lure), and a four-component reference lure. Different letters denote significant difference between treatments ($P < 0.05$). n.c. means no-contact Figure modified from Paper III.

We further explored other *H. uvarum-based* formulations and techniques for behavioural manipulation of *D. suzukii* (Paper IV and V).

In viticulture, *D. suzukii* causes significant problems in the cultivation of certain soft-skinned red grape varieties. We first demonstrated that *H. uvarum* strongly attracted *D. suzukii* when sprayed on the surface of *Vitis vinifera* grapevine leaves. Saliently, this attractiveness was comparable to the attraction induced by ripe grape berries that were susceptible to *D. suzukii* attack (Figure 11A).

In wind-tunnel assays, we showed that *H. uvarum* culture evoked strong odour-mediated flight behaviour in *D. suzukii* even when tested in µL-amounts and when provided in a background of grapevine leaves. What is more, we demonstrated that *H. uvarum* in combination with insecticides typically used for *D. suzukii* management, did not reduce up-wind flight behavioural attraction in *D. suzukii* towards *H. uvarum* (Figure 11B), and enhanced insecticides efficacy (Paper IV). These results emphasize the potential of yeast-insecticide formulations in new attract-and-kill application methods targeting exclusively green-plant leaves rather than the whole plant and fruit.

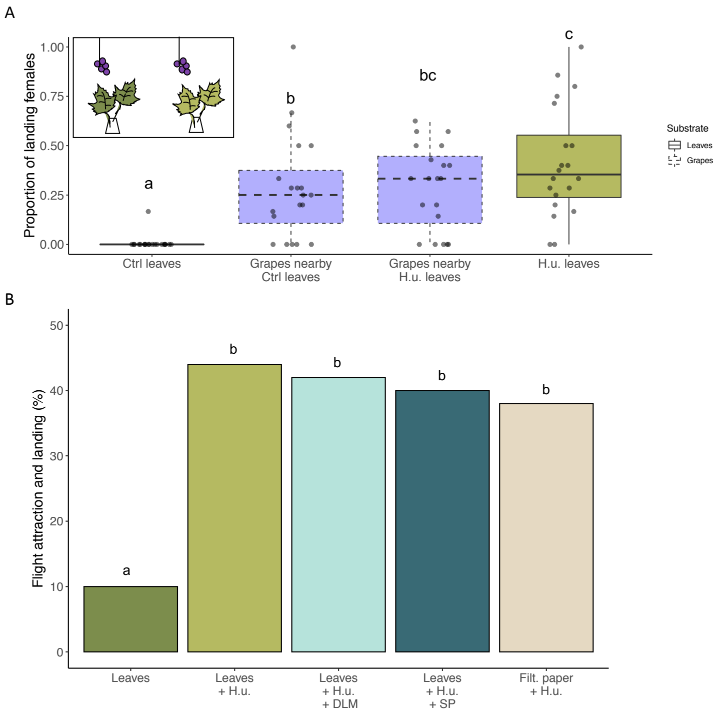

Figure 11. Attractiveness of *Drosophila suzukii* to grapevine leaves sprayed with *Hanseniaspora uvarum* A) Preference of 10 mated *D. suzukii* females when exposed to *Vitis vinifera* leaves treated with *H. uvarum* (light green), untreated leaves (dark green) and grapes (purple) placed nearby the treated and untreated leaves. The boxes represent the interquartile range divided by the median, and whiskers represent the data within 1.5X the interquartile range. Dots represent the data distribution of the individual replicates. Drawing of the experimental design in the top left corner illustrates treated and untreated leaves, and grape berries as tested in the assay. B) Up-wind flight behaviour and landing at the odour source of mated *D. suzukii* females towards headspace volatiles of grapevine leaves (Leaves), leaves sprayed with *H. uvarum* (Leaves + H.u.), leaves sprayed with *H. uvarum* in combination with the insecticides deltamethrin (DLM) or spinosad (SP), and filter paper sprayed with *H. uvarum*. In A and B Different letters above boxes describe significant difference after multiple comparisons of means ($P < 0.05$). Figure modified from Paper IV.

The former laboratory studies provided the basis for the manipulation of *D. suzukii* behaviour in the field. We specifically formulated *H. uvarum* with spinosad insecticide. In a vineyard, we showed that our *H. uvarum*-spinosad formulation, when sprayed to canopy only, reduced fruit infestation by *D. suzukii* at the same rate as the common practice of spraying spinosad to the whole plant and grape berries (Figure 12). More than that, the total amount of spinosad applied in *H. uvarum*-spinosad formulation was ~3 times lower than that in the conventional treatment and prevented spinosad residues on grape berries at harvest (Paper V).

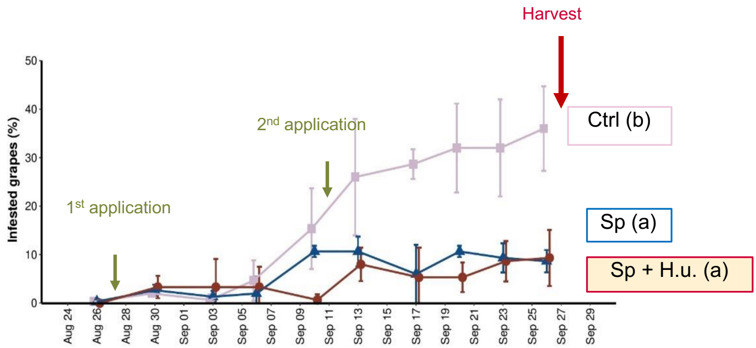

Figure 12. Field evaluation of *Drosophila suzukii* infestation of *Vitis vinifera* grape berries. Efficacy of application of *H. uvarum*-spinosad formulation onto *V. vinifera* canopy (red line, Sp + H.u.), common practice of spinosad application on the whole plant (blue line, Sp), and unsprayed control plants (light purple line, Ctrl). The applied spinosad amounts were 120 g per hectare for the Sp treatment and 36.4 g per hectare for the Sp + H.u. treatment. *Drosophila suzukii* infestation (% infested grapes ± SD) was analysed at ten sampling days during one month before harvest. Treatment names followed by different lowercase letters in brackets denote significant differences in infestation between the treatments ($P < 0.05$). Figure modified from Paper V.

The collective findings illustrate the diversity of possibilities that *H. uvarum* offers to develop novel behaviour-modifying practices to improve *D. suzukii* management. Our results provide routes towards sustainable pest management strategies, in line with previous studies from our and other research groups (Mori et al., 2017; Noble et al., 2019; Bueno et al., 2020; Bianchi et al., 2020). Finally, these results exhibit the tool-box and predictive value of laboratory studies, and the important interplay between fundamental and applied research.

6. Concluding remarks

Drosophila suzukii has gained research attention for the infestation of soft-skinned fruit and fast intercontinental distribution, that made it become an economically significant pest worldwide. Although a lot of studies have shown the wide host range as well as various management strategies to control *D. suzukii*, relatively few studies have based these on sensory ecology and behavioural responses. Thus, the focus of this PhD study was to investigate the relevance of fruit and microbial-associated cues in the behavioural ecology of *D. suzukii*.

In the first part of this thesis (Part I), I describe behavioural responses of *D. suzukii* to fruit cues. I have demonstrated that *D. suzukii* host choice is modulated by mating and fruit ripeness. Specifically, I provided evidence that odour-guided behaviours are of importance for *D. suzukii* host selection and egg-laying decisions. However, complex behaviours such as oviposition rely on the interplay between sensory modalities. Yet, further research is needed to unravel the complexity of *D. suzukii* decision-making within its distinctive chemical space.

In the following part (Part II), I investigated *D. suzukii* attraction and interaction with its closely associated yeast *H. uvarum*, in the context of the fruit host. I have demonstrated that *D. suzukii* responded strongly to *H. uvarum* volatiles. Remarkably, we further demonstrated that *D. suzukii – H. uvarum* interaction shapes the shared fruit habitat in a mutualistic relation. Whether this mutualistic interaction has contributed to *D. suzukii* geographical expansion, or whether other yeasts may play a similar role in the ecology of *D. suzukii*, clearly requires more investigation.

Finally, in Part III (Papers III-V), my aim was to exploit the strong attraction of *D. suzukii* to *H. uvarum* metabolites for manipulating *D. suzukii* behaviour in future pest management. We have demonstrated that different

H. uvarum-based formulations were highly attractive to *D. suzukii* in laboratory and field. More than that, we have provided novel methods to complement existing management practices by manipulating *D. suzukii* behaviour towards more sustainable pest management strategies.

References

Allan, S. A. (2018). Behavior-based control of insect crop pests. In: *Insect Behavior: From Mechanisms to Ecological and Evolutionary Consequences* (pp. 309-331), ed by Cordoba-Aguilar, A., Gonzales-Tokman, D., and Gonzalez-Santoyo, I., New York, NY: Oxford University Press Mexico. https://doi.org/10.1093/oso/9780198797500.003.0020

Anton, S., & Rössler, W. (2021). Plasticity and modulation of olfactory circuits in insects. *Cell and Tissue Research*, *383*(1), 149–164. https://doi.org/10.1007/s00441-020-03329-z

Arnó, J., Solà, M., Riudavets, J., & Gabarra, R. (2016). Population dynamics, non-crop hosts, and fruit susceptibility of *Drosophila suzukii* in Northeast Spain. *Journal of Pest Science*, *89*(3), 713–723. https://doi.org/10.1007/s10340-016-0774-3

Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., Gibert, P., Gutierrez, A. P., Hoelmer, K. A., Hutchison, W. D., Isaacs, R., Jiang, Z.-L., Kárpáti, Z., Kimura, M. T., Pascual, M., Philips, C. R., Plantamp, C., Ponti, L., Vétek, G., … Desneux, N. (2015). Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): A global perspective and future priorities. *Journal of Pest Science*, *88*(3), 469–494. https://doi.org/10.1007/s10340-015-0681-z

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., & Kopp, A. (2014). The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1781), 20132840. https://doi.org/10.1098/rspb.2013.2840

Auer, T. O., & Benton, R. (2016). Sexual circuitry in *Drosophila*. *Current Opinion in Neurobiology*, *38*, 18–26. https://doi.org/10.1016/j.conb.2016.01.004

Becher, P. G., Bengtsson, M., Hansson, B. S., & Witzgall, P. (2010). Flying the Fly: Long-range Flight Behavior of *Drosophila melanogaster* to Attractive Odors. *Journal of Chemical Ecology*, *36*(6), 599–607. https://doi.org/10.1007/s10886-010-9794-2

Becher, P. G., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M. C., Hansson, B. S., Piškur, J., Witzgall, P., & Bengtsson, M. (2012). Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Functional Ecology*, *26*(4), 822–828. https://doi.org/10.1111/j.1365-2435.2012.02006.x

Becher, P. G., Hagman, A., Verschut, V., Chakraborty, A., Rozpędowska, E., Lebreton, S., Bengtsson, M., Flick, G., Witzgall, P., & Piškur, J. (2018).

Chemical signaling and insect attraction is a conserved trait in yeasts. *Ecology and Evolution*, *8*(5), 2962–2974. https://doi.org/10.1002/ece3.3905

Begon, M. (1982). Yeast and *Drosophila*. In *The genetics and biology of Drosophila: Vol. 3a* (M. Ashburner, H. L. Carson & J. Thompson, pp. 345–384). Academic Press.

Bellutti, N., Gallmetzer, A., Innerebner, G., Schmidt, S., Zelger, R., & Koschier, E. H. (2018). Dietary yeast affects preference and performance in *Drosophila suzukii*. *Journal of Pest Science*, *91*(2), 651–660. https://doi.org/10.1007/s10340-017-0932-2

Benton, R. (2015). Multigene Family Evolution: Perspectives from Insect Chemoreceptors. *Trends in Ecology & Evolution*, *30*(10), 590–600. https://doi.org/10.1016/j.tree.2015.07.009

Bianchi, F., Spitaler, U., Castellan, I., Cossu, C. S., Brigadoi, T., Duménil, C., Angeli, S., Robatscher, P., Vogel, R. F., Schmidt, S., & Eisenstecken, D. (2020). Persistence of a Yeast-Based (*Hanseniaspora uvarum*) Attract-and-Kill Formulation against *Drosophila suzukii* on Grape Leaves. *Insects*, *11*(11), 810. https://doi.org/10.3390/insects11110810

Biasazin, T. D., Larsson Herrera, S., Kimbokota, F., & Dekker, T. (2019). Translating olfactomes into attractants: Shared volatiles provide attractive bridges for polyphagy in fruit flies. *Ecology Letters*, *22*(1), 108–118. https://doi.org/10.1111/ele.13172

Billeter, J.-C., & Levine, J. D. (2015). The role of cVA and the Odorant binding protein Lush in social and sexual behavior in *Drosophila melanogaster*. *Frontiers in Ecology and Evolution*, *3*. https://doi.org/10.3389/fevo.2015.00075

Bohbot, J. D., & Pitts, R. J. (2015). The narrowing olfactory landscape of insect odorant receptors. *Frontiers in Ecology and Evolution*, *3*. https://doi.org/10.3389/fevo.2015.00039

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). PNAS Plus: From the Cover: Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences*, *111*(1), E139–E148. https://doi.org/10.1073/pnas.1317377110

Borrero-Echeverry, F., Bengtsson, M., Nakamuta, K., & Witzgall, P. (2018). Plant odor and sex pheromone are integral elements of specific mate recognition in an insect herbivore. *Evolution*, *72*(10), 2225–2233. https://doi.org/10.1111/evo.13571

Bräcker, L. B., Schmid, C. A., Bolini, V. A., Holz, C. A., Prud'homme, B., Sirota, A., & Gompel, N. (2019). Quantitative and Discrete Evolutionary Changes in the Egg-Laying Behavior of Single *Drosophila* Females. *Frontiers in Behavioral Neuroscience*, *13*, 118. https://doi.org/10.3389/fnbeh.2019.00118

Brown, W. L., Eisner, T., & Whittaker, R. H. (1970). Allomones and Kairomones: Transspecific Chemical Messengers. *BioScience*, *20*(1), 21–22. https://doi.org/10.2307/1294753

Budick, S. A., & Dickinson, M. H. (2006). Free-flight responses of *Drosophila melanogaster* to attractive odors. *Journal of Experimental Biology*, *209*(15), 3001–3017. https://doi.org/10.1242/jeb.02305

Bueno, E., Martin, K. R., Raguso, R. A., Mcmullen, J. G., Hesler, S. P., Loeb, G. M., & Douglas, A. E. (2020). Response of Wild Spotted Wing Drosophila (*Drosophila suzukii*) to Microbial Volatiles. *Journal of Chemical Ecology*, *46*(8), 688–698. https://doi.org/10.1007/s10886-019-01139-4

Buser, C. C., Newcomb, R. D., Gaskett, A. C., & Goddard, M. R. (2014). Niche construction initiates the evolution of mutualistic interactions. *Ecology Letters*, *17*(10), 1257–1264. https://doi.org/10.1111/ele.12331

Cardé, R. T., & Haynes, K. F. (2004). Structure of the pheromone communication channel in moths. In R. T. Cardé & J. G. Millar (Eds.), *Advances in Insect Chemical Ecology* (1st ed., pp. 283–332). Cambridge University Press. https://doi.org/10.1017/CBO9780511542664.009

Chandler, J. A., Eisen, J. A., & Kopp, A. (2012). Yeast Communities of Diverse *Drosophila* Species: Comparison of Two Symbiont Groups in the Same Hosts. *Applied and Environmental Microbiology*, *78*(20), 7327–7336. https://doi.org/10.1128/AEM.01741-12

Cloonan, K. R., Abraham, J., Angeli, S., Syed, Z., & Rodriguez-Saona, C. (2018). Advances in the Chemical Ecology of the Spotted Wing Drosophila (*Drosophila suzukii*) and its Applications. *Journal of Chemical Ecology*, *44*(10), 922–939. https://doi.org/10.1007/s10886-018-1000-y

Clyne, P. J., Warr, C. G., Freeman, M. R., Lessing, D., Kim, J., & Carlson, J. R. (1999). A Novel Family of Divergent Seven-Transmembrane Proteins. *Neuron*, *22*(2), 327–338. https://doi.org/10.1016/S0896-6273(00)81093-4

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The Use of Push-Pull Strategies in Integrated Pest Management. *Annual Review of Entomology*, *52*(1), 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407

Crava, C. M., Zanini, D., Amati, S., Sollai, G., Crnjar, R., Paoli, M., Rossi-Stacconi, M. V., Rota-Stabelli, O., Tait, G., Haase, A., Romani, R., & Anfora, G. (2020). Structural and transcriptional evidence of mechanotransduction in the *Drosophila suzukii* ovipositor. *Journal of Insect Physiology*, *125*, 104088. https://doi.org/10.1016/j.jinsphys.2020.104088

Davis, T. S., Crippen, T. L., Hofstetter, R. W., & Tomberlin, J. K. (2013). Microbial Volatile Emissions as Insect Semiochemicals. *Journal of Chemical Ecology*, *39*(7), 840–859. https://doi.org/10.1007/s10886-013-0306-z

de Bruyne, M., & Warr, C. G. (2006). Molecular and cellular organization of insect chemosensory neurons. *BioEssays*, *28*(1), 23–34. https://doi.org/10.1002/bies.20338

Dekker, T., Ibba, I., Siju, K. P., Stensmyr, M. C., & Hansson, B. S. (2006). Olfactory Shifts Parallel Superspecialism for Toxic Fruit in *Drosophila melanogaster* Sibling, *D. sechellia*. *Current Biology*, *16*(1), 101–109. https://doi.org/10.1016/j.cub.2005.11.075

Del Socorro, A. P., Gregg, P. C., Alter, D., & Moore, C. J. (2010). Development of a synthetic plant volatile-based attracticide for female noctuid moths. I. Potential sources of volatiles attractive to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology*, *49*(1), 10–20. https://doi.org/10.1111/j.1440-6055.2009.00733.x

Depetris-Chauvin, A., Galagovsky, D., & Grosjean, Y. (2015). Chemicals and chemoreceptors: Ecologically relevant signals driving behavior in *Drosophila*. *Frontiers in Ecology and Evolution*, *3*. https://doi.org/10.3389/fevo.2015.00041

dos Santos, L. A., Mendes, M. F., Krüger, A. P., Blauth, M. L., Gottschalk, M. S., & Garcia, F. R. M. (2017). Global potential distribution of *Drosophila suzukii* (Diptera, Drosophilidae). *PLOS ONE*, *12*(3), e0174318. https://doi.org/10.1371/journal.pone.0174318

Douglas, A. E. (2007). Symbiotic microorganisms: Untapped resources for insect pest control. *Trends in Biotechnology*, *25*(8), 338–342. https://doi.org/10.1016/j.tibtech.2007.06.003

Douglas, A. E. (2015). Multiorganismal Insects: Diversity and Function of Resident Microorganisms. *Annual Review of Entomology*, *60*(1), 17–34. https://doi.org/10.1146/annurev-ento-010814-020822

Durkin, S. M., Chakraborty, M., Abrieux, A., Lewald, K. M., Gadau, A., Svetec, N., Peng, J., Kopyto, M., Langer, C. B., Chiu, J. C., Emerson, J. J., & Zhao, L. (2021). Behavioral and Genomic Sensory Adaptations Underlying the Pest Activity of *Drosophila suzukii*. *Molecular Biology and Evolution*, *38*(6), 2532–2546. https://doi.org/10.1093/molbev/msab048

Dweck, H. K. M., Ebrahim, S. A. M., Kromann, S., Bown, D., Hillbur, Y., Sachse, S., Hansson, B. S., & Stensmyr, M. C. (2013). Olfactory Preference for Egg Laying on Citrus Substrates in *Drosophila*. *Current Biology*, *23*(24), 2472–2480. https://doi.org/10.1016/j.cub.2013.10.047

Dweck, H. K. M., Ebrahim, S. A. M., Retzke, T., Grabe, V., Weißflog, J., Svatoš, A., Hansson, B. S., & Knaden, M. (2018). The Olfactory Logic behind Fruit Odor Preferences in Larval and Adult *Drosophila*. *Cell Reports*, *23*(8), 2524–2531. https://doi.org/10.1016/j.celrep.2018.04.085

Dweck, H. K. M, Talross, G. J., Wang, W., & Carlson, J. R. (2021). Evolutionary shifts in taste coding in the fruit pest *Drosophila suzukii*. *ELife*, *10*, e64317. https://doi.org/10.7554/eLife.64317

Edgecomb, R. S., Harth, C. E., & Schneiderman, A. M. (1994). Regulation of feeding behavior in adult *Drosophila melanogaster* varies with feeding regime and nutritional state. *Journal of Experimental Biology*, *197*(1), 215–235. https://doi.org/10.1242/jeb.197.1.215

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of "Lure and Kill" in Long-Term Pest Management and Eradication of Invasive Species. *Journal of Economic Entomology*, *102*(3), 815–835. https://doi.org/10.1603/029.102.0301

El-Sayed, A. M., Suckling, D. M., Wearing, C. H., & Byers, J. A. (2006). Potential of Mass Trapping for Long-Term Pest Management and Eradication of Invasive Species. *Journal of Economic Entomology*, *99*(5), 1550–1564. https://doi.org/10.1093/jee/99.5.1550

Fishilevich, E., Domingos, A. I., Asahina, K., Naef, F., Vosshall, L. B., & Louis, M. (2005). Chemotaxis Behavior Mediated by Single Larval Olfactory Neurons in *Drosophila*. *Current Biology*, *15*(23), 2086–2096. https://doi.org/10.1016/j.cub.2005.11.016

Foster, S. P., & Harris, M. O. (1997). Behavioral manipulation methods for insect pest-management. *Annual Review of Entomology*, *42*(1), 123–146. https://doi.org/10.1146/annurev.ento.42.1.123

Gonzalez, F., Borrero-Echeverry, F., Jósvai, J. K., Strandh, M., Unelius, C. R., Tóth, M., Witzgall, P., Bengtsson, M., & Walker, W. B. (2020). Odorant receptor phylogeny confirms conserved channels for sex pheromone and host plant signals in tortricid moths. *Ecology and Evolution*, *10*(14), 7334–7348. https://doi.org/10.1002/ece3.6458

González-Robles, I. W., Estarrón-Espinosa, M., & Díaz-Montaño, D. M. (2015). Fermentative capabilities and volatile compounds produced by *Kloeckera/Hanseniaspora* and *Saccharomyces* yeast strains in pure and mixed cultures during Agave tequilana juice fermentation. *Antonie van Leeuwenhoek*, *108*(3), 525–536. https://doi.org/10.1007/s10482-015-0506-3

Green, J. E., Cavey, M., Médina Caturegli, E., Aigouy, B., Gompel, N., & Prud'homme, B. (2019). Evolution of Ovipositor Length in *Drosophila suzukii* Is Driven by Enhanced Cell Size Expansion and Anisotropic Tissue Reorganization. *Current Biology*, *29*(12), 2075-2082.e6. https://doi.org/10.1016/j.cub.2019.05.020

Gregg, P. C., Greive, K. A., Del Socorro, A. P., & Hawes, A. J. (2010). Research to realisation: The challenging path for novel pest management products in Australia. *Australian Journal of Entomology*, *49*(1), 1–9. https://doi.org/10.1111/j.1440-6055.2009.00732.x

Grosjean, Y., Rytz, R., Farine, J.-P., Abuin, L., Cortot, J., Jefferis, G. S. X. E., & Benton, R. (2011). An olfactory receptor for food-derived odours promotes male courtship in *Drosophila*. *Nature*, *478*(7368), 236–240. https://doi.org/10.1038/nature10428

Haldane, J. (1955). Animal communication and the origin of human language. *Science Progress*, *43*(171), 385–401.

Hamby, K. A., & Becher, P. G. (2016). Current knowledge of interactions between *Drosophila suzukii* and microbes, and their potential utility for pest management. *Journal of Pest Science*, *89*(3), 621–630. https://doi.org/10.1007/s10340-016-0768-1

Hamby, K. A., Hernández, A., Boundy-Mills, K., & Zalom, F. G. (2012). Associations of Yeasts with Spotted-Wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in Cherries and Raspberries. *Applied and Environmental Microbiology*, *78*(14), 4869–4873. https://doi.org/10.1128/AEM.00841-12

Hatano, E., Saveer, A. M., Borrero-Echeverry, F., Strauch, M., Zakir, A., Bengtsson, M., Ignell, R., Anderson, P., Becher, P. G., Witzgall, P., & Dekker, T. (2015). A herbivore-induced plant volatile interferes with host plant and mate location in moths through suppression of olfactory signalling pathways. *BMC Biology*, *13*(1), 75. https://doi.org/10.1186/s12915-015-0188-3

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

Heuskin, S., Verheggen, FJ., Haubruge, É., Wathelet, J.-P., & Lognay, G. (2011). The use of semiochemical slow-release devices in integrated pest management strategies. *Biotechnol Agron Soc*, *15*, 459–470.

Hildebrand, J. G., & Shepherd, G. M. (1997). Mechanisms of olfactory discrimination: Converging Evidence for Common Principles Across Phyla. *Annual Review of Neuroscience*, *20*(1), 595–631. https://doi.org/10.1146/annurev.neuro.20.1.595

Joseph, R. M., & Carlson, J. R. (2015). *Drosophila* Chemoreceptors: A Molecular Interface Between the Chemical World and the Brain. *Trends in Genetics*, *31*(12), 683–695. https://doi.org/10.1016/j.tig.2015.09.005

Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. *Yamanashi Agricultural Experimental Station, Kofu, Review of Applied Entomology*, *29*(622).

Karageorgi, M., Bräcker, L. B., Lebreton, S., Minervino, C., Cavey, M., Siju, K. P., Grunwald Kadow, I. C., Gompel, N., & Prud'homme, B. (2017). Evolution of Multiple Sensory Systems Drives Novel Egg-Laying Behavior in the Fruit Pest *Drosophila suzukii*. *Current Biology*, *27*(6), 847–853. https://doi.org/10.1016/j.cub.2017.01.055

Keesey, I. W., Knaden, M., & Hansson, B. S. (2015). Olfactory Specialization in *Drosophila suzukii* Supports an Ecological Shift in Host Preference from Rotten to Fresh Fruit. *Journal of Chemical Ecology*, *41*(2), 121–128. https://doi.org/10.1007/s10886-015-0544-3

Khallaf, M. A., Auer, T. O., Grabe, V., Depetris-Chauvin, A., Ammagarahalli, B., Zhang, D.-D., Lavista-Llanos, S., Kaftan, F., Weißflog, J., Matzkin, L. M., Rollmann, S. M., Löfstedt, C., Svatoš, A., Dweck, H. K. M., Sachse, S., Benton, R., Hansson, B. S., & Knaden, M. (2020). Mate discrimination among subspecies through a conserved olfactory pathway. *Science Advances*, *6*(25), eaba5279. https://doi.org/10.1126/sciadv.aba5279

Kienzle, R., Groß, L. B., Caughman, S., & Rohlfs, M. (2020). Resource use by individual *Drosophila suzukii* reveals a flexible preference for oviposition into healthy fruits. *Scientific Reports*, *10*(1), 3132. https://doi.org/10.1038/s41598-020-59595-y

Knight, A. L., Basoalto, E., Yee, W., Hilton, R., & Kurtzman, C. P. (2016). Adding yeasts with sugar to increase the number of effective insecticide classes to manage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in cherry: Adding yeast and sugar to manage spotted-wing *drosophila*. *Pest Management Science*, *72*(8), 1482–1490. https://doi.org/10.1002/ps.4171

Knight, A. L., & Witzgall, P. (2013). Combining Mutualistic Yeast and Pathogenic Virus—A Novel Method for Codling Moth Control. *Journal of Chemical Ecology*, *39*(7), 1019–1026. https://doi.org/10.1007/s10886-013-0322-z

Koerte, S., Keesey, I. W., Easson, M. L. A. E., Gershenzon, J., Hansson, B. S., & Knaden, M. (2020). Variable dependency on associated yeast communities influences host range in *Drosophila* species. *Oikos*, *129*(7), 964–982. https://doi.org/10.1111/oik.07180

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., Maradona, M. P., … Herman, L. (2019). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: Suitability of taxonomic units notified to EFSA until March 2019. *EFSA Journal*, *17*(7). https://doi.org/10.2903/j.efsa.2019.5753

Kwadha, C. A., Okwaro, L. A., Kleman, I., Rehermann, G., Revadi, S., Ndlela, S., Khamis, F. M., Nderitu, P. W., Kasina, M., George, M. K., Kithusi, G. G., Mohamed, S. A., Lattorff, H. M. G., & Becher, P. G. (2021). Detection of the spotted wing drosophila, *Drosophila suzukii*, in continental sub-Saharan Africa. *Journal of Pest Science*, *94*(2), 251–259. https://doi.org/10.1007/s10340-021-01330-1

Law, J. H., & Regnier, F. E. (1971). Pheromones. *Annual Review of Biochemistry*, *40*(1), 533–548. https://doi.org/10.1146/annurev.bi.40.070171.002533

Lebreton, S., Borrero-Echeverry, F., Gonzalez, F., Solum, M., Wallin, E. A., Hedenström, E., Hansson, B. S., Gustavsson, A.-L., Bengtsson, M., Birgersson, G., Walker, W. B., Dweck, H. K. M., Becher, P. G., & Witzgall, P. (2017). A *Drosophila* female pheromone elicits species-specific long-range attraction via an olfactory channel with dual specificity for sex and food. *BMC Biology*, *15*(1), 88. https://doi.org/10.1186/s12915-017-0427-x

Lebreton, S., Grabe, V., Omondi, A. B., Ignell, R., Becher, P. G., Hansson, B. S., Sachse, S., & Witzgall, P. (2015). Love makes smell blind: Mating suppresses pheromone attraction in *Drosophila* females via Or65a olfactory neurons. *Scientific Reports*, *4*(1), 7119. https://doi.org/10.1038/srep07119

Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, *67*(11), 1358–1367. https://doi.org/10.1002/ps.2225

Linz, J., Baschwitz, A., Strutz, A., Dweck, H. K. M., Sachse, S., Hansson, B. S., & Stensmyr, M. C. (2013). Host plant-driven sensory specialization in *Drosophila erecta*. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1760), 20130626. https://doi.org/10.1098/rspb.2013.0626

Little, C. M., Chapman, T. W., & Hillier, N. K. (2020). Plasticity Is Key to Success of *Drosophila suzukii* (Diptera: Drosophilidae) Invasion. *Journal of Insect Science*, *20*(3), 5. https://doi.org/10.1093/jisesa/ieaa034

Liu, H. M., Guo, J. H., Cheng, Y. J., Luo, L., Liu, P., Wang, B. Q., Deng, B. X., & Long, C. A. (2010). Control of gray mold of grape by *Hanseniaspora uvarum* and its effects on postharvest quality parameters. *Annals of Microbiology*, *60*(1), 31–35. https://doi.org/10.1007/s13213-010-0018-3

Ljunggren, J., Borrero-Echeverry, F., Chakraborty, A., Lindblom, T. U. T., Hedenström, E., Karlsson, M., Witzgall, P., & Bengtsson, M. (2019). Yeast Volatomes Differentially Affect Larval Feeding in an Insect Herbivore. *Applied and Environmental Microbiology*, *85*(21), e01761-19. https://doi.org/10.1128/AEM.01761-19

Maia, M. F., & Moore, S. J. (2011). Plant-based insect repellents: A review of their efficacy, development and testing. *Malaria Journal*, *10*(S1), S11. https://doi.org/10.1186/1475-2875-10-S1-S11

Mansourian, S., Enjin, A., Jirle, E. V., Ramesh, V., Rehermann, G., Becher, P. G., Pool, J. E., & Stensmyr, M. C. (2018). Wild African *Drosophila melanogaster* Are Seasonal Specialists on Marula Fruit. *Current Biology*, *28*(24), 3960-3968.e3. https://doi.org/10.1016/j.cub.2018.10.033

Markow, T. A., & O'Grady, P. (2006). *Drosophila: A guide to species identification and use*. Academic Press (Elsevier), London.

Masse, N. Y., Turner, G. C., & Jefferis, G. S. X. E. (2009). Olfactory Information Processing in *Drosophila*. *Current Biology*, *19*(16), R700–R713. https://doi.org/10.1016/j.cub.2009.06.026

Mestre, M. V., Maturano, Y. P., Gallardo, C., Combina, M., Mercado, L., Toro, M. E., Carrau, F., Vazquez, F., & Dellacassa, E. (2019). Impact on Sensory and Aromatic Profile of Low Ethanol Malbec Wines Fermented by Sequential Culture of *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* Native

Yeasts. *Fermentation*, *5*(3), 65. https://doi.org/10.3390/fermentation5030065
Miller, J. R., & Gut, L. J. (2015). Mating Disruption for the 21st Century: Matching Technology With Mechanism. *Environmental Entomology*, *44*(3), 427–453. https://doi.org/10.1093/ee/nvv052
Mori, B. A., Whitener, A. B., Leinweber, Y., Revadi, S., Beers, E. H., Witzgall, P., & Becher, P. G. (2017). Enhanced yeast feeding following mating facilitates control of the invasive fruit pest *Drosophila suzukii*. *Journal of Applied Ecology*, *54*(1), 170–177. https://doi.org/10.1111/1365-2664.12688
Noble, R., Dobrovin-Pennington, A., Phillips, A., Cannon, M. F. L., Shaw, B., & Fountain, M. T. (2019). Improved insecticidal control of spotted wing drosophila (*Drosophila suzukii*) using yeast and fermented strawberry juice baits. *Crop Protection*, *125*, 104902. https://doi.org/10.1016/j.cropro.2019.104902
Phaff, H. J., Miller, M. W., Recca, J. A., Shifrine, M., & Mrak, E. M. (1956). Yeasts Found in the Alimentary Canal of *Drosophila*. *Ecology*, *37*(3), 533–538. https://doi.org/10.2307/1930176
Qin, X., Xiao, H., Cheng, X., Zhou, H., & Si, L. (2017). *Hanseniaspora uvarum* prolongs shelf life of strawberry via volatile production. *Food Microbiology*, *63*, 205–212. https://doi.org/10.1016/j.fm.2016.11.005
Raguso, R. A. (2008). Wake Up and Smell the Roses: The Ecology and Evolution of Floral Scent. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 549–569. https://doi.org/10.1146/annurev.ecolsys.38.091206.095601
Raguso, R. A., Agrawal, A. A., Douglas, A. E., Jander, G., Kessler, A., Poveda, K., & Thaler, J. S. (2015). The raison d’être of chemical ecology. *Ecology*, *96*(3), 617–630. https://doi.org/10.1890/14-1474.1
Ramasamy, S., Ometto, L., Crava, C. M., Revadi, S., Kaur, R., Horner, D. S., Pisani, D., Dekker, T., Anfora, G., & Rota-Stabelli, O. (2016). The Evolution of Olfactory Gene Families in *Drosophila* and the Genomic Basis of chemical-Ecological Adaptation in *Drosophila suzukii*. *Genome Biology and Evolution*, *8*(8), 2297–2311. https://doi.org/10.1093/gbe/evw160
Reisenman, C. E., Lei, H., & Guerenstein, P. G. (2016). Neuroethology of Olfactory-Guided Behavior and Its Potential Application in the Control of Harmful Insects. *Frontiers in Physiology*, *7*. https://doi.org/10.3389/fphys.2016.00271
Rodriguez-Saona, C. R., & Stelinski, L. L. (2009). Behavior-Modifying Strategies in IPM: Theory and Practice. In R. Peshin & A. K. Dhawan (Eds.), *Integrated Pest Management: Innovation-Development Process: Volume 1* (pp. 263–315). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8992-3_11

Rundle, H. D., & Nosil, P. (2005). Ecological speciation: Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Scheidler, N. H., Liu, C., Hamby, K. A., Zalom, F. G., & Syed, Z. (2015). Volatile codes: Correlation of olfactory signals and reception in *Drosophila*-yeast chemical communication. *Scientific Reports*, *5*(1), 14059. https://doi.org/10.1038/srep14059

Shah, P. A., & Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, *61*(5–6), 413–423. https://doi.org/10.1007/s00253-003-1240-8

Spitaler, U., Bianchi, F., Eisenstecken, D., Castellan, I., Angeli, S., Dordevic, N., Robatscher, P., Vogel, R. F., Koschier, E. H., & Schmidt, S. (2020). Yeast species affects feeding and fitness of *Drosophila suzukii* adults. *Journal of Pest Science*, *93*(4), 1295–1309. https://doi.org/10.1007/s10340-020-01266-y

Stensmyr, M. C., Dekker, T., & Hansson, B. S. (2003). Evolution of the olfactory code in the *Drosophila melanogaster* subgroup. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1531), 2333–2340. https://doi.org/10.1098/rspb.2003.2512

Stensmyr, M. C., Dweck, H. K. M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S., Wicher, D., Sachse, S., Knaden, M., Becher, P. G., Seki, Y., & Hansson, B. S. (2012). A Conserved Dedicated Olfactory Circuit for Detecting Harmful Microbes in *Drosophila*. *Cell*, *151*(6), 1345–1357. https://doi.org/10.1016/j.cell.2012.09.046

Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: A rewiew. *Cell and Tissue Research*, *275*(1), 3–26. https://doi.org/10.1007/BF00305372

Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J. C., Choi, M.-Y., Cloonan, K., Crava, C. M., Daane, K. M., Dalton, D. T., Diepenbrock, L., Fanning, P., … Walton, V. M. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A Decade of Research Towards a Sustainable Integrated Pest Management Program. *Journal of Economic Entomology*, *114*(5), 1950–1974. https://doi.org/10.1093/jee/toab158

Tasin, M., Bäckman, A.-C., Bengtsson, M., Ioriatti, C., & Witzgall, P. (2006). Essential host plant cues in the grapevine moth. *Naturwissenschaften*, *93*(3), 141–144. https://doi.org/10.1007/s00114-005-0077-7

Turlings, T. C., Loughrin, J. H., McCall, P. J., Röse, U. S., Lewis, W. J., & Tumlinson, J. H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences*, *92*(10), 4169–4174. https://doi.org/10.1073/pnas.92.10.4169

Visser, J. H. (1986). Host Odor Perception in Phytophagous Insects. *Annual Review of Entomology*, *31*(1), 121–144. https://doi.org/10.1146/annurev.en.31.010186.001005

Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A., & Axel, R. (1999). A Spatial Map of Olfactory Receptor Expression in the *Drosophila* Antenna. *Cell*, *96*(5), 725–736. https://doi.org/10.1016/S0092-8674(00)80582-6

Vosshall, L. B., & Stocker, R. F. (2007). Molecular Architecture of Smell and Taste in *Drosophila*. *Annual Review of Neuroscience*, *30*(1), 505–533. https://doi.org/10.1146/annurev.neuro.30.051606.094306

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O'Neal, S. D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. *Journal of Integrated Pest Management*, *2*(1), G1–G7. https://doi.org/10.1603/IPM10010

Wang, Q., Xu, P., Sanchez, S., Duran, P., Andreazza, F., Isaacs, R., & Dong, K. (2021). Behavioral and physiological responses of *Drosophila melanogaster* and *D. suzukii* to volatiles from plant essential oils. *Pest Management Science*, *77*(8), 3698–3705. https://doi.org/10.1002/ps.6282

Wilson, E. O. (1970). Chemical communication within animal species. In *Chemical Ecology* (Sondheimer, E. and Simeone, J.B., pp. 133–155). Academic Press.

Witzgall, P., Kirsch, P., & Cork, A. (2010). Sex Pheromones and Their Impact on Pest Management. *Journal of Chemical Ecology*, *36*(1), 80–100. https://doi.org/10.1007/s10886-009-9737-y

Witzgall, P., Proffit, M., Rozpedowska, E., Becher, P. G., Andreadis, S., Coracini, M., Lindblom, T. U. T., Ream, L. J., Hagman, A., Bengtsson, M., Kurtzman, C. P., Piskur, J., & Knight, A. (2012). "This is not an Apple"–Yeast Mutualism in Codling Moth. *Journal of Chemical Ecology*, *38*(8), 949–957. https://doi.org/10.1007/s10886-012-0158-y

Witzgall, P., Stelinski, L., Gut, L., & Thomson, D. (2008). Codling Moth Management and Chemical Ecology. *Annual Review of Entomology*, *53*(1), 503–522. https://doi.org/10.1146/annurev.ento.53.103106.093323

Wright, R. H. (1964). After Pesticides—What? *Nature*, *204*(4954), 121–125. https://doi.org/10.1038/204121a0

Yousef, M., Aranda-Valera, E., & Quesada-Moraga, E. (2018). Lure-and-infect and lure-and-kill devices based on *Metarhizium brunneum* for spotted wing Drosophila control. *Journal of Pest Science*, *91*(1), 227–235. https://doi.org/10.1007/s10340-017-0874-8

Zars, T. (2000). Behavioral functions of the insect mushroom bodies. *Current Opinion in Neurobiology*, *10*(6), 790–795. https://doi.org/10.1016/S0959-4388(00)00147-1

Popular science summary

Vinegar *Drosophila* flies, you might have experienced them as nuisance in your kitchen when fruit turns overripe, have served as model organism in life science for example to study behaviours of importance for survival and reproduction. Flies, as most insects, heavily rely on their sense of smell and taste to perceive chemicals (such as from overripe fruit) to find their way to food, to choose suitable mating partners, or good places to lay their eggs. Flies sense odorants in the environment mainly by their antennae, which has a similar function as our nose. Thus, the antennal "nose" tells flies about relevant odours in their landscape and they respond accordingly.

Obviously, not all *Drosophila* live in the surrounding of our kitchens, and one specifically is causing troubles in orchards because it happily feeds and breeds in fresh ripening fruit, making them unmarketable. As a result, farmers have experienced yield and economic losses. Its common name is Spotted Wing *Drosophila* (SWD), because males have one dark spot on each wing. Its scientific name is *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae). Understanding its biology, behaviour and ecology is important to design suitable strategies to control the crop damage SWD causes: *knowledge is power*.

SWD prefers different ripening stages of fruit based on the distinct scents that each ripening fruit emits, such us unripe, semi-ripe, ripe and overripe. Interestingly, virgin and mated SWD females differ in their preference for fruit odours. While virgin females are less picky, mated females are strongly attracted to ripe fruit odours, suggesting that their "nose" is guiding them to a suitable fruit source for laying their eggs (Paper I).

Moreover, the SWD "nose" has the ability to detect also microbial odours. As an example, yeast odours might indicate a valuable food source and therefore be very attractive for the flies. On the other hand, odours from mouldy fungi may signal “this is not good”. Notably, SWD and a specific yeast associated with fresh fruit can team-up to inhibit the growth of mould on the fruit, while enhancing both fly and yeast growth in a so-called mutualistic relationship (Paper II).

Evidently, one might ask, *how can we use this information to reduce SWD infestation?* One possibility is to take profit of the super attractive odours emitted by the yeast. In other words, we could use the yeast volatiles to call the *D. suzukii’s* “nose” attention and lure the flies for example into fatal traps. Yeast-based formulations are an effective tool for managing *D. suzukii* in horticulture. More than that, they can help to reduce precautionary insecticide applications and insecticide residues on our precious fruit crops (Papers III-V).

Populärvetenskaplig sammanfattning

Bananfruktflugor (*Drosophila*), de små flugorna du besvärats av i ditt kök när frukt blir övermogen, har varit modellorganismer för att studera viktiga beteenden såsom överlevnad och reproduktion. Flugor, såsom de flesta insekter, är väldigt beroende av sina smak- och doftsinnen för att kunna tolka kemiska signaler (såsom dofter från övermogen frukt) för att hitta till mat, lämpliga parningspartners eller bra platser att lägga sina ägg på. Flugor känner dofter i sin närmiljö främst med sina antenner, vilka kan sägas ha en liknande funktion som våra näsor. Således, så kan flugans "näsa" hjälpa dom att sålla och förstå relevanta dofter i deras omgivning och korrekt utvärdera var de t.ex. bör lägga sina ägg.

Självklart så lever inte alla *Drosophila* i miljöer såsom våra kök, en art är speciellt besvärlig och orsakar allvarliga skador i frukt- och bärodlingar då den lägger sina ägg i färsk mognande frukt, där dess larver gör frukten osäljbar. Resultatet kan bli svåra ekonomiska förluster då den säljbara skörden kan minska drastiskt för odlare. På svenska har inte denna art, *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae), ännu fått ett namn, men kan kännas igen på den prick som hanarna har på varje vinge. Att förstå dess biologi, beteende och ekologi är viktigt för att kunna utveckla lämpliga växtskyddsstrategier: kunskap är nyckeln till detta.

Drosophila suzukii föredrar frukt i vissa mognadsstadier allt beroende på den specifika doftprofil som en omogen, halvmogen, mogen och övermogen frukt avger. Vad som är intressant är att oparade och parade D. suzukii honor har olika preferenser när det gäller dessa fruktdofter. Medans oparade honor är mindre kräsna, så är parade honor väldigt attraherade till dofterna av mognande frukt, vilket pekar på betydelsen av att deras "näsa" leder dom till en lämplig plats att lägga sin ägg på (artikel 1).

Vidare, så har *D. suzukiis* “näsa” förmågan att känna dofter som härrör från mikrobiell aktivitet. Som ett exempel: jästdofter kan indikera på en viktig matkälla och kan därför vara väldigt attraktiva för flugorna. Å andra sidan så kan dofter från mögelsvampar signalera något negativt och skrämma bort flugorna. Intressant nog så lever *D. suzukii* och vissa specifika jästarter associerade med färsk frukt i ett mutualistiskt förhållande som tillsammans minskar mögeltillväxten på frukten, samtidigt som både tillväxttakten hos flugan och jästen ökar (artikel II).

Med all denna kunskap, så frågar man sig, *hur kan vi använda denna information för att minska skadetrycket från D. suzukii*? En möjlighet är att nyttja de väldigt attraktiva dofterna som vissa jästarter avger. Med andra ord så kan vi använda jästdofter för att t.ex. lura *D. suzukii* in i dödliga fällor. Just jästbaserade formuleringar som doftlockbeten är ett effektivt verktyg för att kontrollera *D. suzukii* i frukt- och bärodlingar. Men kanske än viktigare är att användandet av sådana doftlockbeten kan hjälpa oss att minska mängden insekticider, detta till gagn för både odlare och konsumenter (artiklar III-V).

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"It´s a long wild ride
And sometimes we're tired
When we reach our destination
But we're always glad to be here"

M.B.

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"Hoy no es siempre" y "Pobre eres si no llevas repletas las arcas de tu corazón"
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Acta Universitatis agriculturae Sueciae
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The spotted wing drosophila, *Drosophila suzukii*, is a worldwide spreading pest of soft fruit and berries. This thesis demonstrates: (*i*) that *D. suzukii* host choice is modulated by fruit ripeness and fly mating, (*ii*) a reciprocal niche construction and mutualistic interaction between *D. suzukii* and the yeast *Hanseniaspora uvarum* on fresh fruit, (*iii*) the *D. suzukii-H. uvarum* association can be exploited for the development of lures to monitor and control the invasive pest.

Guillermo Rehermann Del Rio completed his graduate education at the Department of Plant Protection Biology, SLU, Alnarp. He received his M.Sc. in Biological Sciences from the Universidad de la República, Montevideo, Uruguay.

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