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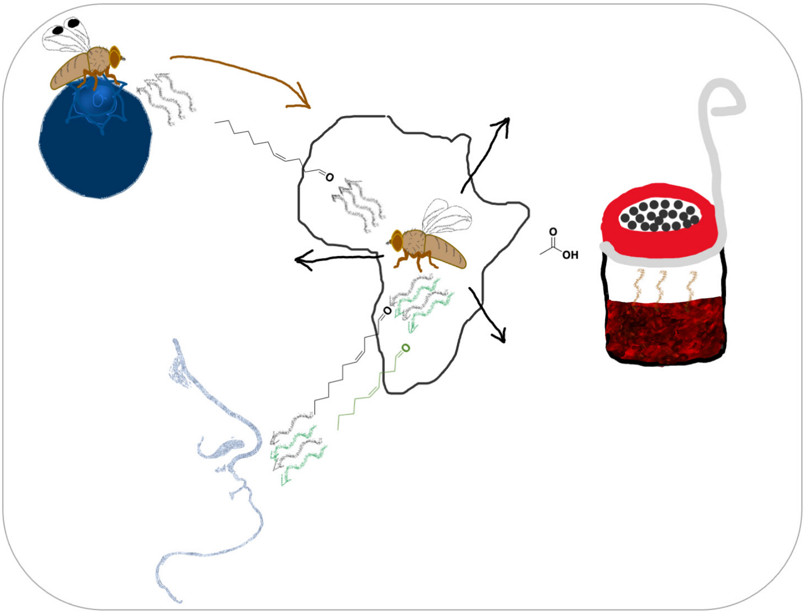
Out of and into Africa

Odour-mediated interaction and detection of the human commensal *Drosophila melanogaster* and the invasive fly *Drosophila suzukii*

CHARLES ATIENO KWADHA



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Out of and Into Africa: Odour-mediated interaction and detection of the human commensal *Drosophila melanogaster* and the invasive fly *Drosophila suzukii*

Abstract

Living organisms use their sense of smell to discriminate odours. Humans can sense females of the cosmopolitan *Drosophila melanogaster*, but whether humans can discriminate the cosmopolitan *D. melanogaster* strain from the conspecific Zimbabwe strain, was unknown. We showed that the cosmopolitan females emit a pheromonal aldehyde, (4*Z*)-4-undecenal (*Z*4-11Al), while Zimbabwe females emit *Z*4-11Al and (4*Z*)-4-nonenal (*Z*4-9Al), and that humans can discriminate the scent of the two strains. The aldehydes are oxidation products of cuticular dienes. Since across *Drosophila* species dienes have biological activities and can, during oxidization produce aldehydes, we propose that the aldehydes are involved in reproductive isolation. Another part of the thesis addresses a closely related species of *D. melanogaster*, *D. suzukii*, which is an invasive pest that damages soft fruits and causes financial losses. Its distribution in Sub-Saharan Africa was unknown. Traps targeting *D. suzukii* often attract non-target species such as *D. melanogaster*. We showed that *D. suzukii* is present in Kenya and that a mutualistic yeast, *Hanseniaspora uvarum*, can improve selectivity of traps. Curiously, on fruit, presence of *D. melanogaster* induces oviposition avoidance in *D. suzukii.* We showed that *D. suzukii* is present in Kenya and that a mutualistic yeast, *Hanseniaspora uvarum,* can improve selectivity of traps. We established that *Z*4-11Al partly induces the avoidance observed in *D. suzukii*. Altogether, our findings advance our understanding of the principle of olfaction across phylogenetically distant species, the dispersal of *D. suzukii* in Africa, and the potential of *H. uvarum* and chemo-ecological interactions in enhancing sound management of *D. suzukii*.

Keywords: olfaction, semiochemicals, human commensal, *Drosophila,* egg-laying, heterospecific interaction.

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Kutoka na Kuingia Afrika: Muingiliano na ugunduaji wa *Drosophila melanogaster* na spishi vamizi *Drosophila suzukii* kwa harufu

Ikisiri

Viumbehai, kupitia kwa hisia ya harufu, vinaweza kutambua makemikali yenye manufaa ya kiikolojia. Wanadamu wanahisi wadudu wanawake wa *Drosophila melanogaster* (cosmopolitan) wapatikanapo na wanadamu. Mwituni Afrika ya Kati, kunayo kikundi kingine cha *D. melanogaster*, Zimbabwe. Tunaripoti kuwa wanawake wa vikundi hivi viwili, wanatofautiana kwa harufu. Cosmopolitan wanatoa (4*Z*)-4-undecenal ilhali Zimbabwe wanatoa (4*Z*)-4-undecenal na (4*Z*)-4-nonenal. Makemikali yanayojulikana kama pheromones hasa aldehydes na yanatolewa kufuatia kuvunjika kwa hidrokaboni wa khitini za *Drosophila.* Tofauti huu inasababisha wanadamu kubainisha vikundi hivi kwa kunusa. Bali na utafiti wa awali, tunapendekeza kuwa aldehydes yanahusika kutenganisha uzaaji kwa spishi za *Drosophila.* Kinyume na *D. melanogaster, D. suzukii* inayojulikana kama drosophila yenye madoadoa, ni muharibifu wa matunda laini. Kuwepo kwake Kusini mwa Sahara haikujulikana. Mitego yanotumika kunasa *D. suzukii* yananasa wadudu wasiolengwa kama *D. melanogaster*. Tunaripoti kuwa *D. suzukii* ipo Kenya na tumebaini kuwa *Hanseniasopra uvarum*, chacha inayohusiana na *D. suzukii*, yanauwezo wa kuimarisha mitego dhidi ya *D. suzukii*. Pia, kuwepo kwa *D. melanogaster* inazuia *D. suzukii* kutaga. Tumebaini kuwa (4*Z*)-4-undecenal, ni mojawapo wa kemikali ya *D. melanogaster* inayosababisha kutotaga kwa *D. suzukii*. Hivyo, tumeonyesha kuwa viumbehai vinakanuni moja ya hisia za harufu, enezeaji wa *D. suzukii* na uwezo wa kutumia uhisiano kati ya *D. suzukii* na viumbe vingine kuboresha vita dhidi ya *D. suzukii*.

Dhana muhimu: hisia, kemikali, uhusiano na binadamu, *Drosophila,* maathiriano wa spishi tofauti.

Anwani ya mwandishi: Charles Atieno Kwadha, Chuo Kikuu cha Sayansi za Kilimo cha Uswidi, Idara ya Biolojia ya Ulinzi wa Mimea, Alnarp, Uswidi.

Dedication

To my first and second family.

“If you want to learn, you have to observe nature, all answers lie within.”
Carlos Ruiz Zafon

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. Frey T*, **Kwadha CA***, Haag F, Wallin EA, Holgersson E, Hedenstrom E, Bohman B, Bengtsson M, Becher PG, Krautwurst D, Witzgall P. The human odorant receptor OR10A6 is tuned to the pheromone of the commensal fruit fly *Drosophila melanogaster*. Accepted manuscript (*iScience*).

II. **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, 251-259.

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*, 115, 999-1007.

IV. **Kwadha CA**, Rehermann G, Tasso Deni, Fellous S, Bengtsson M, Prud'homme B, Witzgall P, Becher PG. Olfactory detection of *Drosophila melanogaster* pheromone (*Z*4)-4-undecenal causes oviposition avoidance in *Drosophila suzukii*. Manuscript.

V. **Kwadha CA**, Bohman B, Bengtsson M, Witzgall P, Becher PG. Divergence of cuticular hydrocarbons: do oxidation products contribute to reproductive isolation in *Drosophila*?. Manuscript.

*Shared first authorship

The contribution of Charles Atieno Kwadha to the papers included in this thesis was as follows:

I. Designed pheromone collection with PGB, collected, and analyzed pheromone collection with BB, MB and PGB, and wrote the manuscript together with co-authors.

II. Designed fieldwork with PGB, conducted 2019 survey, performed morphological identification, partly analyzed the data, and wrote the manuscript together with co-authors.

III. Contributed in the field work and wrote the manuscript together with co-authors.

IV. Designed the assays with PGB, performed oviposition assays with DT, performed wind-tunnel and electrophysiological assays, performed data analysis, and wrote the manuscript together with co-authors

V. Designed the theme with PGB, analyzed data and wrote the manuscript together with co-authors.

Publications not contained in this thesis

- Naundrup A, Bohman B, **Kwadha CA**, Jensen AB, Becher PG, de Fine Licht HH (2022). Pathogenic fungus uses volatiles to entice male flies into fatal matings with infected female cadavers. *The ISME Journal*, published online, https://doi.org/10.1038/s41396-022-01284-x

- Cattaneo AM, Witzgal P, Bengtsson M, **Kwadha CA**, Becher PG, Walker WB. Heterologous expression and functional characterization of *Drosophila suzukii* OR69a splice forms unveiled response to kairomones and to one candidate pheromone. Submitted manuscript.

Abbreviations

5,9-HD	(5*Z*,9*Z*)-5,9-heptacosadiene
7,11-HD	(7*Z*,11*Z*)-7,11-heptacosadiene
AL	Antennal lobe
CHs	Cuticular hydrocarbons
*c*VA	*cis*-11-vaccenyl acetate
FID	Flame ionization detector
GC-EAD	Gas Chromatography coupled to Electroantennographic Detection
GC-MS	Gas Chromatography- Mass Spectrometry
LH	Lateral horn
OB	Olfactory bulb
OBPs	Odorant binding proteins
ODEs	Odorant degrading enzymes
OE	Olfactory epithelium
*Orco*3	Odorant co-receptor mutant line 3
ORs	Olfactory receptors
OSNs	Olfactory sensory neurons
SWD	Spotted wing drosophila
WT3	Wild-type 3
*Z*4-11Al	(4*Z*)-4-undecenal
*Z*4-9Al	(4*Z*)-9-nonenal

1. Introduction

Insects represent the predominant group of living organisms on Earth, as reflected in the sheer taxonomic diversity i.e., vast number of genera and species, and ecological functionalities (Schowalter, 2016). To humans, the insect diversity is critical since different species provide various ecological services, for example pollination and nutrient cycling. Besides, humans are also stunned by negative impacts attributable to some insects such as agricultural pests (Morris, 2020; Schowalter, 2016). Across genera, insect species exhibit an extraordinary ability to adapt to diverse environmental conditions, a feature which has contributed to their dominance on Earth (Schowalter, 2016). For instance, in the genus *Drosophila*, species are distributed across tropical, semi-tropical and temperate regions with deserts, plains, swamps, savannah, woodlands, and forests playing habitats to them (Throckmorton, 1975).

The advancement of *Drosophila* species out of the native tropical areas into a wide range of habitats is characterized by the availability and use of varying nutritional resources. Evolutionary pressure shaped divergence of *Drosophila* species and the use of fermenting substrates as well as fresh plant tissues, saps, fleshy fungi, and fruits as hosts (Markow *et al.*, 2008; Throckmorton, 1975). These substrates not only became food sources for *Drosophila* but also their rendezvous and ovipositing sites, and home for their offspring (Markow *et al.*, 2008). Worth noting, while adults of most *Drosophila* species feed on multiple food substrates, oviposition and larval development occurs on more confined substrates. During host expansion, some *Drosophila* species such as *D. melanogaster* (Meigen), became generalists, ovipositing and inhabiting diverse substrates. However, some species such as *D. sechellia* (Tsacas and Baechli) became specialists which oviposit and inhabit only specific substrates, in this case *Morinda citrifolia*

(Linnaeus) (Markow *et al.*, 2008; Lachaise *et al.*, 1988). Such adaptive diversification might have contributed to an emergence of a mutualistic association of drosophilid flies with bacteria and yeasts (Chakraborty *et al.*, 2022; Janson *et al.*, 2008).

Just like for other insects, survival of Drosophila flies relies on their ability to locate and select conspecifics for mating, hosts for feeding and egg-laying (Markow *et al.*, 2008). Additionally, avoiding competition and threats from natural enemies are important survival traits. One thing that characterises all those life aspects of *Drosophila* flies is the emission of volatile compounds. Perception and decoding ecologically relevant information allow adaptive responses, an inference can thus be made that the life of a fly is full of odour-mediated behaviour. The discoveries of insect pheromones and olfactory receptors (ORs) opened doors for studies that led to understanding of mechanisms behind olfaction (Buck *et al.*, 1991; Regnier *et al.*, 1968). Part of these discoveries are directly connected to the chemical ecology of *Drosophila*. For example, studies on *Drosophila* pheromone communication have led to the establishment of cuticular hydrocarbons that are used as sex pheromones and chemosensory receptors that enable recognition of the pheromones (Khallaf *et al.*, 2021; Benton, 2007).

Drosophila species are distinct in the production of cuticular compounds and chemical signals. Not surprisingly, therefore, humans have capitalized on this to foster understanding of chemical ecology of *Drosophila* species, which could improve our knowledge of human-*Drosophila* commensalism. Interestingly, humans can detect the scent of the commensal *D. melanogaster*. Beside, knowledge of chemical ecology of *Drosophila* can define our management strategies against pest species.

This thesis has utilized an integrated methodological approach consisting of behavioural, sensory, and chemical analyses to decipher if humans can discriminate between cosmopolitan and Zimbabwe female flies of *D. melanogaster*. Moreover, similar methodological approaches were used to advance our understanding of the distribution of the invasive fly, *D. suzukii*, and odour-mediated *D. melanogaster*- *D. suzukii* interactions.

2. Background

2.1 Olfaction in animals

2.1.1 Overview

Olfaction – the sense of smell – is vital for all animals. It provides any animal with ability to perceive and respond through an adaptive behaviour to the surrounding chemical signals. For terrestrial animals such as humans and most insects, the signals are air-borne (Ache *et al.*, 2005). Chemical signals are varying and often complex both in mixtures and coded information. Besides, the signals are often carried in air current, meaning signals are sporadically perceived. Altogether, these complexities presents animals with a challenge to decode encrypted messages (Ache *et al.*, 2005; Hildebrand *et al.*, 1997). As such, to survive, species are expected to adapt the olfactory system dependent on specific ecological needs.

But still, the olfactory systems between phylogenetically distant vertebrate and invertebrate species share organizational resemblance, which according to Ache and Young (2005) is routed back to the shared challenges. A case example is seen in humans and *Drosophila*. Regardless of the morphological dissimilarities, both human nose and *Drosophila* antennae (Fig. 1 and 2) show converging functional adaptation right from the onset of encoding chemical signals to the higher-order processing of the signals (Ache *et al.*, 2005; Hildebrand *et al.*, 1997).

2.1.2 Olfaction in *Drosophila*

Drosophila flies have two peripheral olfactory organs, the antennae which is the primary olfactory organ, and the maxillary palps, a mouthpart with gustatory and olfactory function (Figure 1). The organs contain sensory hair-like structures known as sensilla that form the smallest *Drosophila* sensory units. In the antennae, there are three morphologically distinct sensilla types i.e., basiconic, coeloconic and trichoid, distributed on the third antennal segment. The maxillary palps contain two sensilla types, basiconic and trichoid sensilla (Shanbhag *et al.*, 1999; Carlson, 1996).

Every sensillum houses up to four olfactory sensory neurons (OSNs) that expresses a seven-transmembrane domain odorant receptor (OR) and odorant co-receptor (*Orco*). There are pores on each sensillum (Figure 1) which interface the sensilla with their interior components allowing passage of chemical odorants adsorbed by waxy cuticle into sensillum lymph. Present in the lymph are water soluble proteins i.e. the odorant degrading enzymes (ODEs) which degrade odorant molecules, and the odorant binding proteins (OBPs) (Schmidt *et al.*, 2020; Vogt, 2003). There exists evidence that OBPs have various functionalities. One such function is dissolution and transportation of odour molecules through the lymph to ORs (Vosshall, 2000; Stengl *et al.*, 1999). But as Schmidt and Benton discuss (2020), the biochemical mechanisms underlying the functionalities remain unclear.

Once bound to ORs, information coded in the molecules triggers signal transduction followed by channeling through axons of OSNs to the antennal lobe (AL) of the fly's brain (Figure 1). The *Drosophila* AL is organized into various morphologically and functionally distinct glomeruli (Grabe *et al.*, 2016). Even though a given OR can be expressed in different OSNs, such OSNs are projected into a single glomerulus (Laissue *et al.*, 2008). The information is further transmitted via projection neurons (PNs) into the higher brain center of the fly, the mushroom bodies (MBs) and the lateral horns (LHs) (Figure 1). While the mushroom bodies process odorants that mediate learned behavioral responses, LHs process odorants such as pheromones and kairomones that drive innate behaviours (Schultzhaus *et al.*, 2017). The *Drosophila* kairomone and pheromone tuned ORs are expressed in OSNs housed in the basiconic or trichoid sensilla respectively (Laissue *et al.*, 2008; van Naters *et al.*, 2007).

However, Lebreton et al., (2017) intriguingly observed that two *D. melanogaster Or*69a isoforms (*Or*69aA and *Or*69aB), expressed in the basiconic sensillum ab9, have a dual affinity to food and pheromone odorants. With the ab9 expressing an OR with pheromone affinity, it challenges the previously proposed concept that pheromones are detected by single classes of OSNs expressed in trichoid sensilla (Laissue *et al.*, 2008; Hallem *et al.*, 2006; Hildebrand *et al.*, 1997). It remains to be established if there are other *Drosophila* ORs expressed in basiconic and trichoid sensilla, with dual affinity.

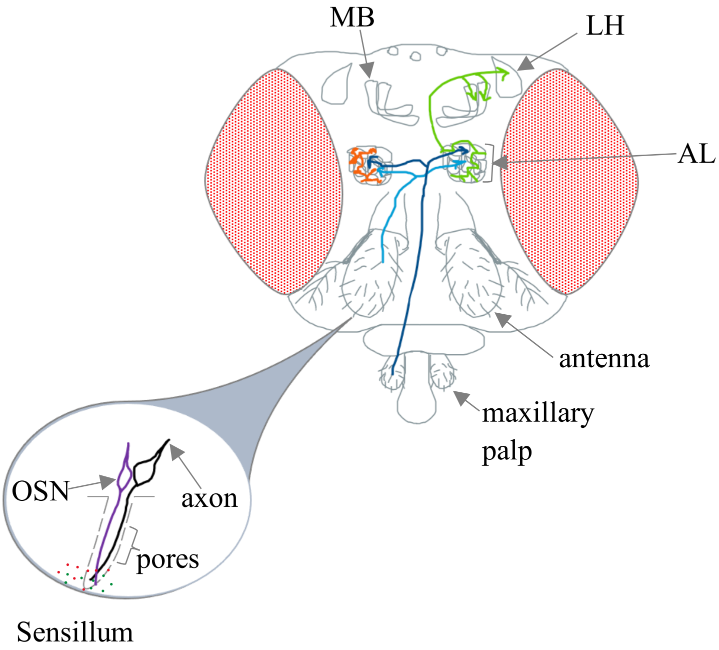

Figure 1. A schematic presentation of odorant detection and transduction into the higher brain centers of *Drosophila*. The red and green dots represent odorants that bind with ORs expressed in olfactory sensory neurons (OSN). The sky blue and dark blue arrows show neurons transmitting signals into the antennal lobe (AL) which contains glomeruli. The orange and green lines represent lateral interneurons and projection neurons respectively. Odorant information is interpreted in the mushroom body (MB) and lateral horns (LH) of the brain. Modified from Perisse *et al.*, (2017).

2.1.3 Olfaction in humans

In humans, the hierarchy of the sense of smell relative to other senses is debatable (Lundstrom *et al.*, 2011; Shepherd, 2004). However, the role that olfaction plays in human physiology and pleasure such as in food preferences, mother-child bonding, and mood cannot be overlooked (Lundstrom *et al.*, 2011; Pinto, 2011). Generally, humans are regarded as microsmatic compared to other mammals such as rodents that are macrosmatic, because the olfactory system is highly developed in rodents relative to humans (Ache *et al.*, 2005; Shepherd, 2004; Price, 2003). For instance, humans have fewer olfactory genes than rodents. But as Shepherd (2004) argues, the low number of ORs in humans should not be obviously correlated with low sensitivity to smell.

The human nose is the peripheral olfactory organ (Figure 2). There are two routes through which humans can perceive odorant molecules i.e., through the orthonasal and retronasal routes. The orthonasal route allows passage of odorants in the inhaled air whereas the retronasal route permits sensation of flavors attributed to ingested food and liquids (Pinto, 2011; Shepherd, 2004). The nasal cavity has at its apex, an olfactory epithelium (OE) which contains OSNs (Figure 2). All OSNs have dendrites extending as olfactory cilia into the mucosal layer. Just like in *Drosophila*, OSNs express ORs. However, just like in mice, every human OSN is believed to express a single OR in humans as opposed to *Drosophila*, in which a single OSN can co-express up to three ORs (Pinto, 2011; Laissue *et al.*, 2008; Ache *et al.*, 2005). The OE is covered with a mucosal layer, produced by the Bowman's gland. The mucous, equivalent to the *Drosophila* sensillum lymph, contains soluble proteins i.e. OBPs, that are non-homologous to *Drosophila* proteins (Hildebrand *et al.*, 1997).

Odorant molecules diffuse in mucous and through the olfactory cilia, are then transported by OBPs to the ORs. Odorant-OR binding invoke neuronal signals, which are carried by axons of OSNs to the glomeruli of the olfactory bulb (OB) (Figure 2). A given OSN expressing the same OR synapses onto the same glomerulus (Sharma *et al.*, 2019; Pinto, 2011; Shipley *et al.*, 2003), implying that signals generated by all OSNs sensitive to a specific odorant are transmitted into the same glomerulus. From the glomeruli, the signals are sent to the olfactory cortex and further to higher cortical regions including hypothalamus and limbic areas (Sharma *et al.*, 2019; Pinto, 2011; Shipley *et al.*, 2003).

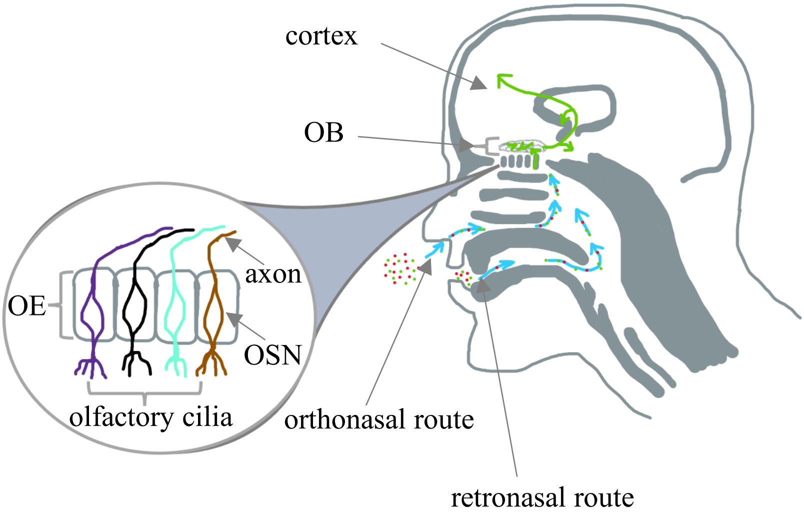

Figure 2. A schematic presentation of olfactory pathway in humans. The red and green dots represent odorant molecules that gate into the olfactory system through orthonasal and retronasal routes, indicated by sky blue arrows. The molecules through olfactory cilia extending from the olfactory epithelium (OE), bind with ORs expressed in olfactory sensory neurons (OSN). The olfactory bulb (OB) contains glomeruli. The green arrows represent glomeruli neurons projecting onto olfactory cortex and other cortical areas. Modified from Sharma *et al.*, (2019).

2.2 Chemical signals and cues in *Drosophila*

2.2.1 Overview

For *Drosophila*, ecologically relevant chemical molecules originate from the flies themselves, food and oviposition resources, natural enemies as well as associated microbes. From drosophilids, it could be conspecific or heterospecific molecules. If the emitted molecules induce a specific response in another *Drosophila* fly of the same species, it is considered a pheromone (Yew *et al.*, 2015; Wyatt, 2014; Karg *et al.*, 1999). Molecules released by host fruits and fruit-associated microbes intermix. The molecules are utilized as cues for locating suitable, nutrient-rich and safe food or oviposition resources. Collectively, they are fruit and microbial semiochemicals (Stensmyr *et al.*, 2012; Karg *et al.*, 1999; Starmer *et al.*, 1986).

Regardless of the emitter, some chemical molecules can trigger a response in a receiver *Drosophila.* However, there are molecules that despite triggering a response, production of the molecules are not biochemically selected by the emitting organism to invoke a response, in such cases, the molecules are referred to as chemical cues. On the other hand, some molecules are selected and released because of the stereotyped response such molecules trigger in the receiver, hence termed chemical signals (Wyatt, 2014).

2.2.2 Pheromones

Drosophila pheromones are produced in oenocyte cells of the cuticle and male ejaculatory bulbs. In both sexes, oenocytes produce cuticular hydrocarbons (CHs) some of which have pheromonal functions. Meanwhile, *cis*-11-vaccenyl acetate (*c*VA) is produced by ejaculatory bulbs of mature males (Ferveur *et al.*, 1997; Blomquist *et al.*, 1987). Pheromone signals can play a multifunctional role, a phenomenon described as pheromone parsimony (Blomquist *et al.*, 2010; Blum, 1996). For instance, in *D. melanogaster*, the male produced pheromone, *c*VA, functions as an aggregation and aggression pheromone. In a synergistic role, *c*VA together with food cues aggregate flies. However, for naïve males, *c*VA acts as aggression stimulating pheromone. While *c*VA stimulates attraction of females to males, females carrying *c*VA after copulation are less attractive to prospective male partners (Lebreton *et al.*, 2012; Amrein, 2004; Ferveur, 1997). For oviposition site seeking flies, CH pheromones deposited on a host, for example by an egg-laying *D. melanogaster*, indicates to the conspecifics, a suitable oviposition site (Duménil *et al.*, 2016).

Moreover, one of the in-depth studied pheromonal role is sexual communication in *Drosophila*. Species specific volatile and semi-volatile signals are involved in mate recognition and discrimination, a choreographic process entailing orientation, tapping, wing vibration, licking and copulation (Mazzoni *et al.*, 2013; Ferveur, 1997; Spieth, 1974). Divergence of signals and behavioural traits accompanied geographical range expansion of *D. melanogaster* (see section 2.3). A case example is the divergence of pheromones in cosmopolitan and Zimbabwe *D. melanogaster* populations, which modulate discriminatory mating behaviour between the populations. For instance, in cosmopolitan *D. melanogaster*, females produce a CH component, (7*Z*,11*Z*)-7,11-heptacosadiene (7,11-HD) which induces excitatory behaviour in males. In the Zimbabwe population, female releases (5*Z*,9*Z*)-5,9-heptacosadiene (5,9-HD) that stimulate males (Ferveur *et al.*, 1996). But as Lebreton *et al.* (2017) showed, 7,11-HD is prone to oxidation, in which (4*Z*)-4-undecenal (*Z*4-11Al) is produced. Relative to 7,11-HD perceived from a few centimetres away, *Z*4-11Al is perceived by males from a relatively longer distance than 7,11-HD (Borrero-Echeverry *et al.*, 2022; Lebreton *et al.*, 2017).

2.2.3 Fruit and microbial semiochemicals

Plant hosts are food sources, rendezvous sites and egg-laying sites for *Drosophila*. There are diverse plant parts used as hosts (see section 1). Although *D. melanogaster* is a generalist species, it only uses overripe and fermenting fruit substrates, from which volatile chemical cues such as acetic acid, 2-phenylethanol, and ethyl acetate (amongst others) are released. The cues provide a guiding plume utilized by *Drosophila* to locate the substrates (Zhu *et al.*, 2003; Reed, 1938). Besides, *Drosophila* flies have a mutual association with yeasts, in which the developing larvae of flies benefits nutritionally while yeast get dispersed by the flies (Starmer *et al.*, 1986). Such mutualistic yeasts also emit volatiles that enhances attraction of flies to the substrates. For instance, volatile headspace emitted from grape juice inoculated with *Hanseniaspora uvarum* was more attractive than non-inoculated grape juice (Palanca *et al.*, 2013; Chandler *et al.*, 2012).

On the flip side, wounded hosts can be associated with mould that emit warning signals. *Penicillium expansum* (Link), associated with decaying apples, produces a pungent earthy smell of geosmin (Mattheis *et al.*, 1992). Geosmin antagonizes attraction of *Drosophila* flies to food related cues and induces oviposition aversion (Stensmyr *et al.*, 2012; Becher *et al.*, 2010; Mattheis *et al.*, 1992). Intriguingly, geosmin-producing *Streptomyces* species inhibit growth of *Saccharomyces cerevisiae* (Arndt *et al.*, 1999).

Taken together, host fruits and associated microbes signals are diverse in nature and functionality, perhaps this could partly account for the fact that there are many *Drosophila* ORs tuned to fruit and microbial cues (Laissue *et al.*, 2008).

2.3 Human commensalism and perception of the commensal *Drosophila*

The dispersal of modern humans is traced back to ~150, 000 years ago out of Africa, a source of the current global human distribution (Majumder *et al.*, 2015; Jones *et al.*, 1986). Similarly, *D. melanogaster*, a member of *melanogaster* species subgroup, was endemic to Afrotropical regions but has since spread globally except for the extreme latitudinal and longitudinal regions (Sprengelmeyer *et al.*, 2020; Lachaise *et al.*, 1988). *Drosophila melanogaster* is historically associated with human activities and even found in human buildings, hence the commensalism (Mansourian *et al.*, 2016; Lachaise *et al.*, 2004). Besides, *D. melanogaster* has a short-lifespan, high fecundity, can tolerate a wide temperature range and utilize a wide host range (Keller, 2007), which account for the ubiquitous presence of a once forest-dwelling fly.

Despite the human commensalism and *D. melanogaster* being an intensively studied model organisms, it was not until 2018 that the first study showed that humans can perceive the scent of the fly with high sensitivity. Members of a sensory panel were able to discriminate a male from a female fly owing to the distinct scent of the female pheromone, *Z*4-11Al (Becher *et al.*, 2018; Lebreton *et al.*, 2017). Moreover, a cosmopolitan female fly can spoil a wine party since as little as 1 ng *Z*4-11Al, distorts the sensation of wine (Becher *et al.*, 2018). A forthright explanation for the shared perception of the female fly's pheromone with humans remains to be established.

2.4 Application of semiochemicals in *Drosophila* management

Semiochemicals have been used in trapping and manipulating behavior of insect pests. For example, in the codling moth, *Cydia pomonella* (Linnaeus), the sex pheromone codlemone is used for disrupting mating. Food baited traps are used in monitoring and trapping invasive tephritid flies, such as *Bactrocera dorsalis* (Saunders) (Biasazin *et al.*, 2018; Witzgall *et al.*, 2008; Karg *et al.*, 1999). Although *D. melanogaster* is not a pest it is still considered a nuisance and managed for example in private households. Back in 1938, Melia Reed modified a trap previously used by William Barrows. Reed baited the trap with fermenting banana, and later with ethyl alcohol and acetic acid, perhaps the first defined trap to be used for managing *D. melanogaster* (Reed, 1938). Later, other modifications followed. For instance, sticky cards were introduced in traps baited with banana and yeast (Birmingham *et al.*, 2011; Zhu *et al.*, 2003).

Unlike *D. melanogaster*, the spotted wing drosophila (SWD), *D. suzukii* (Matsumura), is an invasive pest of diverse soft fruits that attacks ripening and ripe stages of fruits. *Drosophila suzukii* is native to East Asia but has spread globally (Ørsted *et al.*, 2019; Karageorgi *et al.*, 2017; Kenis *et al.*, 2016; Poyet *et al.*, 2015). Because of the damage on fruits caused by *D. suzukii* and subsequent economic losses, studies aimed at establishing eco-friendly strategies were initiated. Traps baited with wine and vinegar, or a blend of chemical components associated with fermenting fruits, are used for mass trapping and monitoring of *D. suzukii* (Tait *et al.*, 2021; Landolt *et al.*, 2012). Moreover, certain microbe-, plant- and insect-associated compounds with repellent and oviposition aversive effects on *D. suzukii* have been reported (Tait *et al.*, 2021; Cloonan *et al.*, 2018). However, achieving an optimized management of *D. suzukii* using these compounds remains elusive. Partly because some aversive compounds require high doses to remain active in the field. Also, there are concerns over potential effects some compounds could have on fruit flavors and that *D. suzukii* could become resistant to the aversive compounds (Tait *et al.*, 2021).

In attract and kill methods, *D. suzukii* is attracted to a lethal bait. A case example is foliage treatment of grapevine with a combination of Spinosad and yeast. *Hanseniaspora uvarum* is a yeast species not only predominant amongst yeast isolates of *D. suzukii,* but also is highly attractive (Rehermann *et al.*, 2022; Spitaler *et al.*, 2022).

2.5 Research gaps in *Drosophila*

Sexual communication has been extensively studied in *Drosophila* species (Marcillac *et al.*, 2005; Amrein, 2004; Ferveur *et al.*, 1996). With Lebreton *et al.,* (2017) reporting that *Z*4-11Al, an aldehyde produced during oxidation of 7,11-HD, is used in *D. melanogaster* (cosmopolitan) sexual communication, intriguing questions emerged. Do other *Drosophila* flies in a similar way produce aldehydes upon oxidation of cuticular dienes? And if aldehydes were to be produced, are such aldehydes involved in species recognition and discrimination? Do the aldehydes interact with host cues across *Drosophila* species? If yes, does such an interaction play a role in reproductive isolation in *Drosophila* species? Answers to these questions could be informative with respect to *D. suzukii*, which until now has no reported pheromone (Snellings *et al.*, 2018). Moreover, Becher *et al*., (2018) showed that *Z*4-11Al gives a distinct scent to female flies of the cosmopolitan populations. That the females of Afro-populations such as Zimbabwe predominantly produce 5,9-HD than 7,11-HD, suggests that the Zimbabwe population emits a dissimilar aldehyde, and humans might be able to discriminate between the cosmopolitan *D. melanogaster* and Zimbabwe females based on scent.

There are on-going studies to enhance management of *D. suzukii*, as illustrated by recent reviews (Tait *et al.*, 2021; Cloonan *et al.*, 2018). Optimal management requires among other things a better understanding of the distribution of the invasive pest, heterospecific interaction and specificity of baits. Moreover, given that *D. suzukii* has a wide invasive distribution range and that some of the *D. suzukii*-free areas have favorable environmental conditions (Ørsted *et al.*, 2019), distribution and monitoring studies targeting such *D. suzukii*-free regions such as the continental Sub-Saharan Africa are needed. Previous trapping of *D. suzukii* with baited traps revealed co-occurrence of *D. suzukii* with other *Drosophila* flies, such as *D. melanogaster*. But the presence of *D. melanogaster* induces oviposition avoidance in *D. suzukii* (Shaw *et al.*, 2018), suggesting a role of either microbial or pheromonal cues related to *D. melanogaster*. Therefore, deciphering such cues is essential. Additionally, baits as currently used, trap non-targeted drosophilids and other arthropods demonstrating the need to enhance bait specificity.

3. Objectives

The general objective of this thesis was to enhance our knowledge of the behavioural relevance of volatile chemical signals and cues to *D. melanogaster* and *D. suzukii*, both from basic and applied perspectives.

The first part of this thesis revisits the scent of the fly and sexual communication in *Drosophila*. The specific objectives addressed were:

i. To establish distinct aldehydes produced by female *D. melanogaster* of a Zimbabwe strain and investigate for the human ability to discriminate between the cosmopolitan and Zimbabwe females based on scent (Paper I).

ii. To determine whether other behaviorally active pheromonal dienes of *Drosophila* species might produce aldehydes during oxidation (Paper V).

The second part of this thesis studies monitoring of *D. suzukii* in Sub-Saharan Africa and specificity of a mutualistic yeast-based lure against *D. suzukii*. The specific objectives addressed were:

i. To find out potential establishment of *D. suzukii* in the continental Sub-Saharan Africa (Paper II).

ii. To investigate the performance of a *H. uvarum* based lure and establish specificity for *D. suzukii* (Paper III).

The third part of this thesis investigates the involvement of *D. melanogaster* pheromones in *D. suzukii* avoidance behaviour. The specific objective was:

i. To establish the effect of a male and female *D. melanogaster* pheromone, *c*VA and *Z*4-11Al respectively, on oviposition behaviour of *D. suzukii* (Paper IV).

4. Methods

4.1 Insects studied

4.1.1 *Drosophila melanogaster*

Two strains of *D. melanogaster*, cosmopolitan (Dalby-SLU Alnarp) and Zimbabwe (S-29, Bloomington), were studied in this thesis. Both were maintained on a standard sugar-yeast-cornmeal diet (Chakraborty *et al.*, 2022) at room temperature (RT) (25 ± 2 °C), relative humidity (RH) 50 ± 5% and a 12:12 (light: darkness) photoperiod. Newly eclosed flies, collected 3-6 h post eclosion, were immobilized and sexed under CO_2. Then kept separately until experimental period.

4.1.2 *Drosophila suzukii*

Two strains of *D. suzukii* were used, *D. suzukii* Italian strain originated from a stock culture collected in Italy (Revadi *et al.*, 2015), and *D. suzukii* wild-type 3 (WT3) originated from Japan (Karageorgi *et al.*, 2017). The odorant co-receptor (*Orco*3) mutant line was generated from *D. suzukii*-WT3 strain using CRISP-cas9 system (Karageorgi *et al.*, 2017). The Italian and WT3 strains were maintained on a similar diet and room conditions as in section 4.1.1. However, *Orco*3 line was reared on a German Food Sick-Fly Formulation (NutriFly®GF).

4.2 Baited trapping, monitoring, and detection

Wine and vinegar-based baits have been widely used in previous trapping and monitoring studies to establish the presence and distribution of *D. suzukii*

(Tait *et al.*, 2021; Landolt *et al.*, 2012). In paper II of this thesis, home-made traps (Figure 3a) baited with wine and vinegar, and commercial traps Red Drososan traps (Koppert Biological Systems) (Figure 3b) were used to determine the presence of *D. suzukii* in soft fruit farms distributed across seven counties in Kenya. In paper III, Red Drososan traps were enriched with *H. uvarum* culture/headspace-based synthetic blend or 80 mL of the commercial Riga bait. The traps were put up at 5 sites on Alnarp Campus (SLU, Sweden) to compare the attractiveness and specificity of *H. uvarum* relative to the Riga bait.

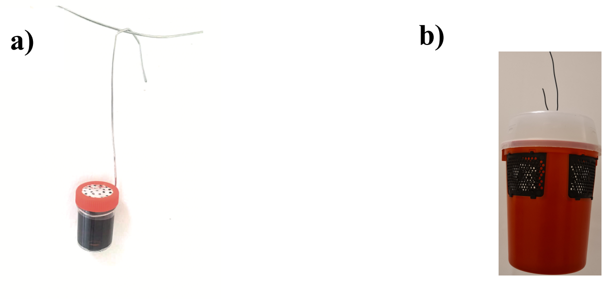

Figure 3. *D. suzukii* monitoring traps: a) Home-made trap baited with apple cider vinegar and red wine, b) Drososan trap baited with commercial lure or yeast.

4.3 Oviposition assays

Oviposition assays are used in *Drosophila* studies as a readout for establishing preference and avoidance of stimuli. Besides, the assays allow the contribution of different sensory modalities involved in oviposition behaviour to be inferred (Karageorgi *et al.*, 2017; Joseph *et al.*, 2009). In paper V, Petri-dish assays (Figure 4a) were used to determine the effect that *D. melanogaster* pheromones have on oviposition behaviour of *D. suzukii*. BugDorm cage assays (Figure 4b) were used in a follow-up study to establish olfactory avoidance of *Z*4-11Al by *D. suzukii* Italian, WT and *Orco*3 strains.

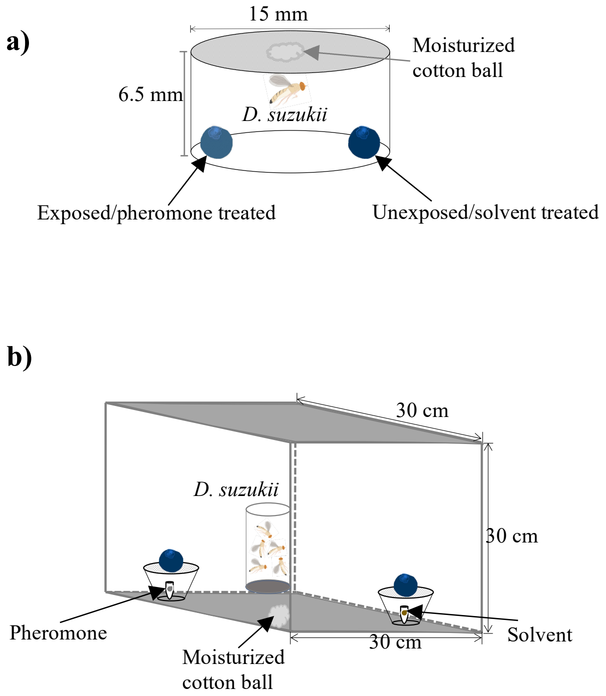

Figure 4. Schematic illustration of oviposition assay set-ups: a) Petri-dish; b) BugDorm cage.

4.4 Wind-tunnel assay

In the wild, *Drosophila* flies locate the source of attractive chemical signals by signal-guided movement. At the same time, aversive chemical cues are avoided, for instance fewer flies get attracted to vinegar with than without geosmin (Becher *et al.*, 2010). Importantly, by coupling of wind-tunnel with a piezo-sprayer, the rate and amount of test cues dissolved in solvents can be stabilized through an assay (Becher *et al.*, 2010). In paper III, a wind tunnel (Figure 5) was utilized to measure *D. suzukii* attraction to *H. uvarum* headspace or a synthetic blend consisting of the chemical headspace components. Similarly, in paper IV, the assay was used to determine the effect of *Z*4-11Al on attraction of *D. suzukii* to *H. uvarum.* In both cases, *D. suzukii* Italian strain was tested.

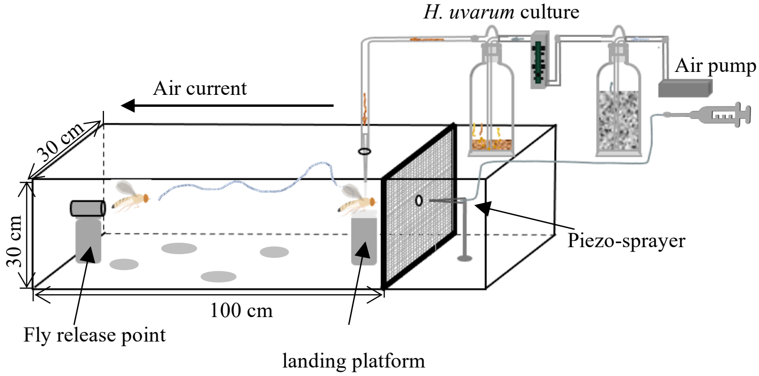

Figure 5. Schematic illustration of a wind-tunnel assay.

4.5 Pheromone collection and analysis

Since 1969 when *c*VA was first reported, solvent extraction has been used to collect pheromonal CHs in *Drosophila*, in which flies or fly tissues are immersed in organic solvent (Antony *et al.*, 1982; Butterworth, 1969). In 2017, Lebreton et al., (2017) used a headspace extraction method whereby moisturized, charcoal-filtered air was blown over flies constrained in a glass capillary. The capillary was then rinsed with solvent to collect headspace extracts. Volatile pheromone components can also be collected using other methods such as thermal desorption and solid phase micro-extraction (SPME) (Dweck *et al.*, 2015; Farine *et al.*, 2012). Chemical profiles of collected samples are analysed in gas chromatography-mass spectrometry (GC-MS) and with the aid of MS libraries and retention indices, pheromone components are identified. In paper I, cosmopolitan and Zimbabwe flies were exposed to glass vials. After release of the flies, the vials were rinsed with solvent under ultrasonic water bathing. The solvent extracts were concentrated to ~5 μL, and 2 μL were analysed by GC-MS.

4.6 Sensory assays

Gas chromatography-electroantennogram detection (GC-EAD) provides an interface to couple analytical chemistry with insect olfaction. In GC-EAD, stimuli components that stimulate olfactory sensilla are simultaneously recorded with a flame ionization detector (FID) peak. Identified components are used as defined synthetic individual compounds or blends in GC-EAD and in screening for behavioral activities. On the other hand, sensory evaluation is used in food and wine industry to characterize products based on senses such as smell (Lawless *et al.*, 2010). In paper I, a triangle test was used to determine the ability of humans to discriminate between females of the cosmopolitan and Zimbabwe strains of *D. melanogaster*. In paper IV, *D. suzukii* Italian, WT3 and *Orco*3 strains were used in GC-EAD recording to find out whether *D. suzukii* olfactory perceives the female *D. melanogaster* pheromone, *Z*4-11Al.

5. Summary of results and discussion

5.1 Pheromone detection of *D. melanogaster*

A sensory panel established that in the background of water and wine, vials with 10 ng *Z*4-11Al resemble vials previously exposed to cosmopolitan female flies (Figure 6A). Analytical comparison of the chemical profiles revealed that the cosmopolitan females emit *Z*4-11Al. To the contrary, Zimbabwe females release both aldehydes with more *Z*4-9Al than *Z*4-11Al (Figure 6 B &C).

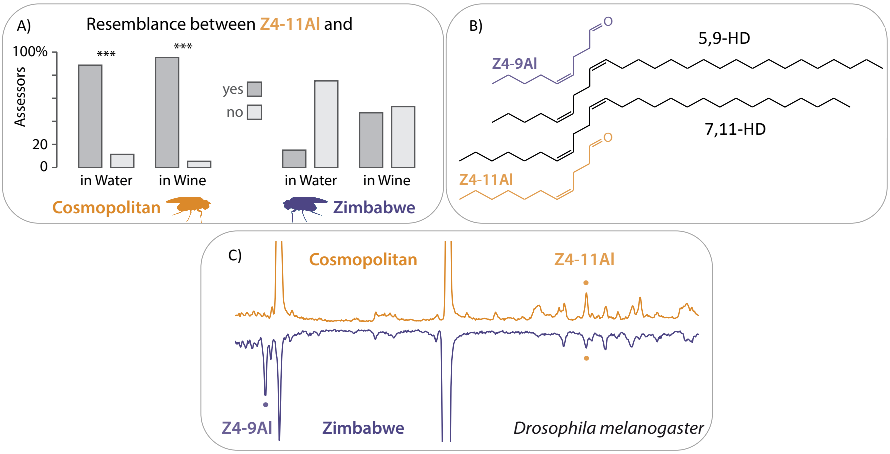

Figure 6. Sensory discrimination between cosmopolitan and Zimbabwe *D. melanogaster* females by humans, and production of *Z*4-11Al and *Z*4-9Al by these two fly strains. (A) Olfactory resemblance (*yes* or *no*) of 10 ng synthetic *Z*4-11Al and the odour of cosmopolitan or Zimbabwe female flies, in water and wine. Asterisks show significant differences between tests with the two fly strains in water and wine, respectively (***$p<0.001$; χ^2-test). (B) 7,11-HD is the hydrocarbon precursor of the cosmopolitan *D. melanogaster* female pheromone *Z*4-11Al. Females of the Zimbabwe strain produce in addition 5,9-HD and the corresponding oxidation product is *Z*4-9Al. (C) Chromatograms of headspace collections from batches of 60 females, with *Z*4-9Al and *Z*4-11Al highlighted. Zimbabwe flies produce *Z*4-9Al in a 2.6±0.7-fold amount, compared to *Z*4-11Al (n=10). Adopted from Paper I.

In the subsequent tests, panelists found that vials exposed to cosmopolitan flies are discriminable from Zimbabwe flies (Figure 7). We then presented synthetics of *Z*4-9Al and *Z*4-11Al individually. While the panelists could still discriminate the compounds, the level of discrimination was not as strong as the fly discrimination test. However, when we blended *Z*4-9Al and *Z*4-11Al (in 10:3 ratio) and tested against *Z*4-11Al alone, the outcome mirrored the fly discrimination (Figure 7). Cuticular hydrocarbons can be transferred to a partner during mating (Everaerts *et al.*, 2010) and we therefore asked if *Z*4-11Al could be transferred to males. Subsequently, we established that humans discriminate between virgin and mated males of the cosmopolitan flies and that mated males had a scent recognizable as *Z*4-11Al. Moreover, the test revealed intriguingly that human sensitivity to the pheromone was even clearer without ethanol solvent as a background odour.

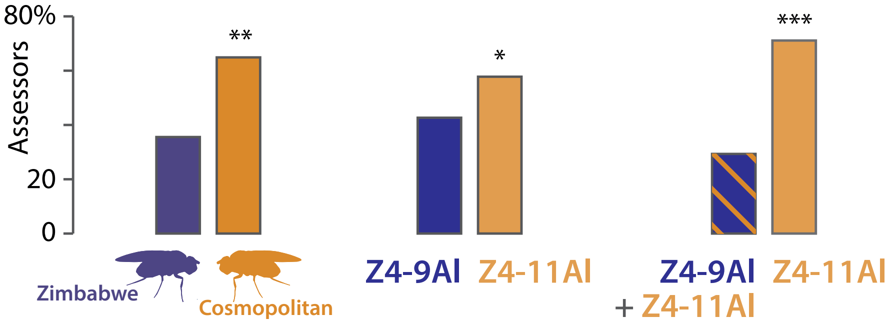

Figure 7. Olfactory resemblance (*yes* or *no*) of cosmopolitan vs Zimbabwe *D. melanogaster* females, of 10 ng Z4-9Al vs 10 ng Z4-11Al, and a 10:3-ng blend of Z4-9Al and Z4-11Al vs 10 ng Z4-11Al. Bars marked with asterisks are significantly different (n=45 panelists, χ^2-test; *$p<0.05$, ** $p<0.01$ and *** $p<0.001$, respectively). Vials containing synthetic compounds and fly odour were filled with 2 mL redistilled water. Adopted from Paper I.

That virgin males of the cosmopolitan and Zimbabwe flies lack the two aldehydes *Z*4-9Al and *Z*4-11Al in their chemical profile corroborates sexual dimorphism in both strains (Grillet *et al.*, 2012; Ferveur, 2005). Emission of the aldehydes by female flies implies possibility of similar oxidation products in females of other *Drosophila* species. Therefore, we revisited sexual communication in *Drosophila*. We reviewed previously reported cuticular dienes with a role in mate recognition and discrimination. We found that across four *Drosophila* subgroups (*melanogaster*, *obscura*, *repleta* and *virilis*), an additional 15 cuticular dienes have been studied in different species. The dienes could be oxidized to produce saturated and unsaturated aldehydes (Figure 8).

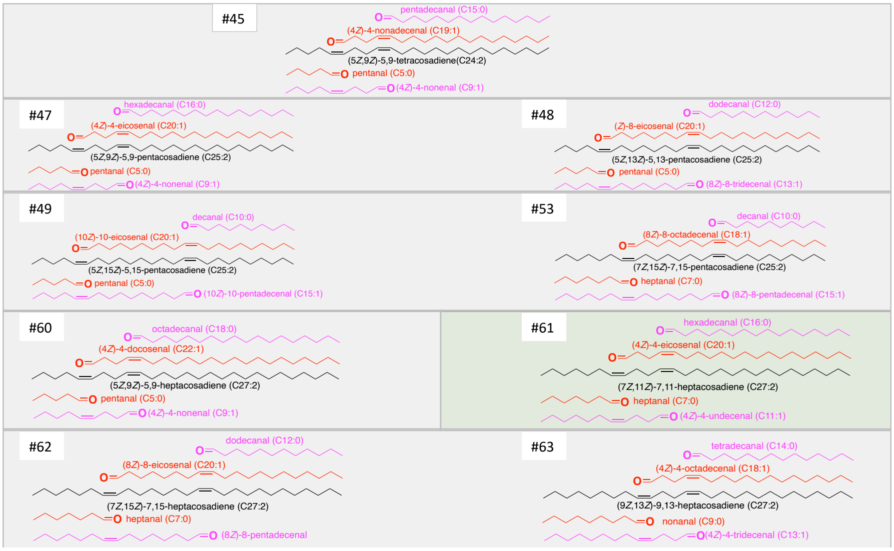

Figure 8. An excerpt from oxidation of *Drosophila* cuticular dienes' chart of paper V. Using *D. melanogaster* diene, #61, that was shown to undergo oxidation (Lebreton *et al.*, 2017) as an example, we show that oxidation of dienes would produce aldehydes. For a complete chart, see paper V.

Altogether, our findings support the concept that cuticular dienes undergoes oxidation. Furthermore, the presence of a more than two-fold amount of *Z*4-9Al to *Z*4-11Al, confirms production of more 5,9-HD than 7,11-HD in Zimbabwe flies (Lebreton *et al.*, 2017; Billeter *et al.*, 2009; Ferveur *et al.*, 1996).

As to whether *Z*4-9Al plays a role in sexual communication remains to be tested. Given that *Z*4-11Al enhances attraction to food and elicit courtship in cosmopolitan flies but not the sibling species *D. simulans* (Borrero-Echeverry *et al.*, 2022; Lebreton *et al.*, 2017), a similar role of *Z*4-9Al in sexual communication of Zimbabwe strain of *D. melanogaster* would be expected. Reflecting on this, we propose that across *Drosophila* species, cuticular dienes are oxidized and produce volatile aldehydes. Further, we propose that the previously reported behavioural activities of the respective dienes, are a collective effect of the dienes and the aldehydes. Finally, we put forward that the aldehydes are involved in reproductive isolation in the genus *Drosophila*.

That humans can consistently perceive small amounts of *Z*4-11Al against the cocktail of odour from wine, indicates that human sense of smell is highly sensitive and discriminative to the pheromone. Thus, our test confirms that a female fly can spoil a glass of wine (Becher *et al.*, 2018). Given that a binary blend of the aldehydes compared to a single aldehyde has a different scent quality perceivable by humans, it hints at how interaction of odorant mixtures with ORs can affect human odour perception (Claire *et al.*, 2020).

Despite the divergent structural organization in the olfactory organs (section 2.1.2 and 2.1.3), the convergent perception of the fly pheromone by flies and human lend support to the idea that the fundamental mechanism of odour perception is shared between invertebrates and vertebrates (Hildebrand *et al.*, 1997).

5.2 Monitoring and detection of *D. suzukii*

In 2019, through monitoring and trapping, we showed the presence of the invasive pest, *D. suzukii,* in one farm in Kenya. A follow-up study in 2020 confirmed the establishment of the pest in the farm (Figure 9). In both periods, traps baited with wine and vinegar, or a commercial lure, captured non-targeted arthropods including other drosophilids.

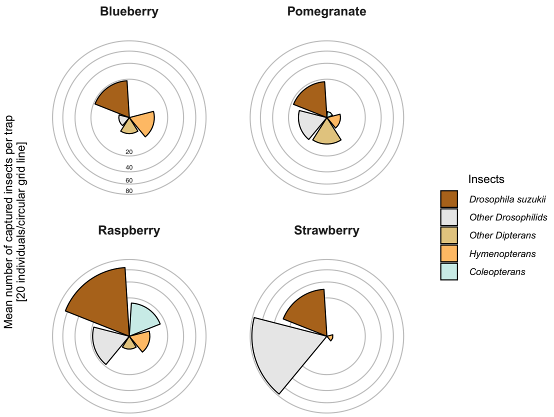

Figure 9. *Drosophila suzukii* monitoring results from a fruit farm in Kenya. Radial plots illustrate the mean number of *D. suzukii*, other drosophilids, other dipterans, hymenopterans and coleopterans per trap caught across four different fruit crops in 2019. The circular grid lines of each radial plot represent 20 captured individuals belonging to different insect categories listed above. Adopted from paper II.

Consequently, there is a need to for a widespread monitoring of *D. suzukii* in the region as well as a need to enhance specificity of traps used against *D. suzukii.* Exploiting existing association with mutualistic microbes, such as *H. uvarum*, could provide an avenue (Rehermann *et al.*, 2022; Spitaler *et al.*, 2022). We compared the performance of Red Drososan traps baited with fresh culture of *H. uvarum* or the commercial Riga lure. We established that while Riga baited traps captured more *D. suzukii* than *H. uvarum*, the relative proportion of *D. suzukii* to other drosophilids was higher in traps baited with *H. uvarum* than Riga lure (Figure 10).

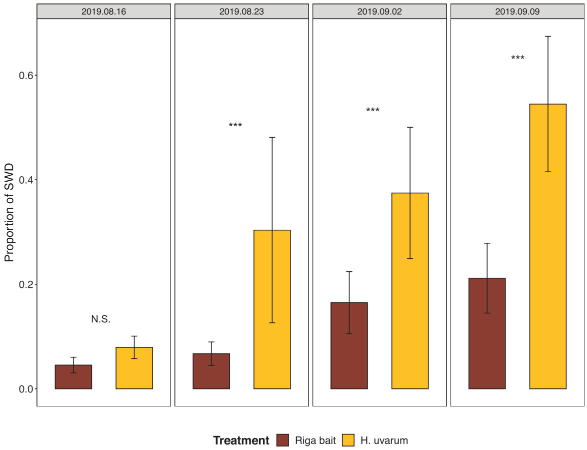

Figure 10. Field trapping Proportion (Mean ± SEM) of *Drosophila suzukii* (SWD) relative to other drosophilids caught with Drososan traps baited with culture of *Hanseniaspora uvarum* or Riga bait. The monitoring was performed in four periods between August and September 2019, at Alnarp, Sweden. The dates give the days when traps were collected from the field after 3 d (for the samples 2019.08.16 and 2019.08.23) or 4 d (samples 2019.09.02 and 2019.09.09) exposure. Asterisks indicate significant difference (***p < 0.001). N.S. indicate no significant difference. Modified from paper III.

With the headspace of *H. uvarum* triggering about 70 and 40 % upwind flight and landing respectively in *D. suzukii*, we analytically characterized *H. uvarum* headspace. A blend consisting of seven synthetic components of the headspace was constituted and used for a follow-up wind tunnel and field survey. A drowning solution consisting of aqueous acetic acid and ethanol was added in baited traps. For the survey, three baits were compared: the seven-component blend, *H. uvarum* headspace and a reference blend consisting of four components (Cha *et al.*, 2013). A comparison of trap capture revealed that while *H. uvarum*- baited traps had the lowest mean capture of *D. suzukii* per trap per day, the traps had the highest proportion of *D. suzukii* relative to other baits. In contrast, the seven-component blend trapped more *D. suzukii* but with less selectivity than the *H. uvarum* culture (Figure 11).

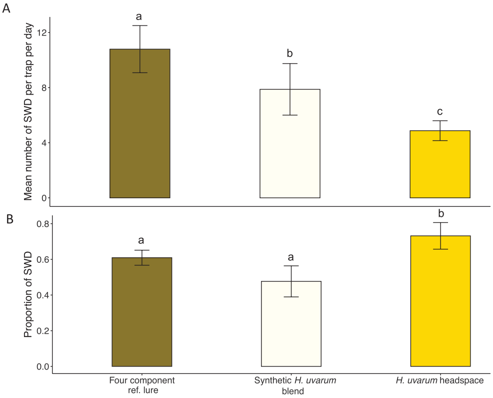

Figure 11. Field trapping with Drososan traps baited with three different lures: *Hanseniaspora uvarum* headspace; a synthetic blend based on *H. uvarum* headspace and a synthetic reference lure. The assay was performed in November 2019, in a wood and shrub-covered area at Alnarp, Sweden (n = 6). (A) Mean number (± SEM) of trapped *Drosophila suzukii* (SWD) per trap per day. (B) Proportion (Mean ± SEM) of SWD relative to other trapped arthropods for each tested lure. Traps baited with *H. uvarum* headspace extract showed a higher specificity for attracting SWD. Different letters indicate significant difference between treatments. Modified from paper III.

In summary, from our monitoring and detection studies in Kenya, we have shown that *D. suzukii* is present and established with a limited distribution in Kenya. The findings corroborate previous predictions that the region has suitable prevailing environmental conditions that can support the invasive *D. suzukii* (Ørsted *et al.*, 2019; dos Santos *et al.*, 2017). Besides, our findings add to the list of African countries invaded by *D. suzukii* (Boughdad *et al.*, 2021; Hassani *et al.*, 2020).

That we observed non-targeted arthropods (including other drosophilids) in our traps, confirmed what was already known that the vinegar-wine and existing commercial lures are non-specific to *D. suzukii*. Hence by-catch demonstrate a limitation of commonly used trapping baits that are based on vinegar and wine (Larson *et al.*, 2021; Cha *et al.*, 2018). With culture of *H. uvarum* trapping the highest proportion of *D. suzukii* relative to other drosophilids and other athropods, confirms reports of both attractiveness and selectivity of *H. uvarum* to *D. suzukii* (Rehermann *et al.*, 2022; Scheidler *et* *al.*, 2015). As our seven-component synthetic blend was not as selective as the *H. uvarum,* it suggests that the blend was sub-optimal and thus requires optimization.

5.3 Odour-mediated heterospecific interaction

Through oviposition and wind-tunnel assays, as well as GC-EAD, we investigated pheromone-mediated interaction between *D. melanogaster* and *D. suzukii*. We showed that pre-exposure of blueberries to 10 flies of male or female *D. melanogaster* induces oviposition avoidance in *D. suzukii,* but that the avoidance effect is more pronounced against female exposed than male exposed berries (Figure 12 A). When a male or a female specific pheromone, *c*VA or *Z*4-11Al respectively, were tested, we established that *Z*4-11Al and not *c*VA induces oviposition avoidance (Figure 12 B).

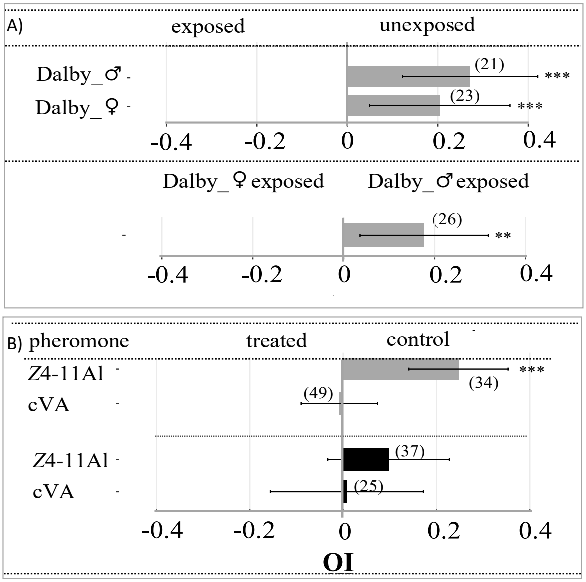

Figure 12. Oviposition indices (OI) for *D. suzukii* in a Petri-dish assay using blueberries exposed to mated *D. melanogaster* (Dalby strain). The bars show the mean OI ± standard error: A) when given a choice between berries exposed to male (♂) or female (♀) *D. melanogaster* vs unexposed berries, and when given a choice between berries exposed to mated male *vs* female *D. melanogaster*. B) when given a choice between berries treated with 5ng Z4-11Al/ cVA *vs* EtOH (gray bars) or berry purée treated with 5ng Z4-11Al/ cVA *vs* EtOH (black bars). Numbers in bracket indicate replicates. Asterisks indicate significant avoidance (*p*<*** 0.001; ** *p*<0.01; **p*<0.05). Modified from Paper IV.

Furthermore, we show that the avoidance is olfactory mediated. Whereby without expression of the *Orco* gene, *D. suzukii* would lay eggs randomly in the presence of *Z*4-11Al. Besides, absence of *Orco* confers no antennal response to *Z*4-11Al in *D. suzukii*. While using *H. uvarum* culture as a background odour in a flight assay, we established that spraying of 10 ng *Z*4-11Al per minute, caused about 20 % reduction in attraction of *D. suzukii*.

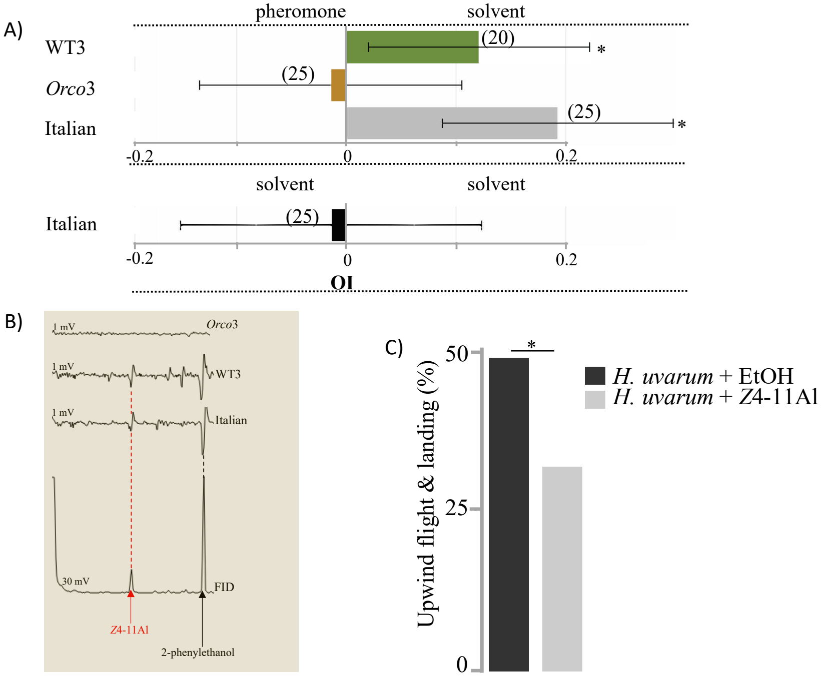

Figure 13. Oviposition, GC-EAD, and flight assay with *D. suzukii*. A) OI for *D. suzukii* lines in 100 µl of EtOH *vs* 100 µl of EtOH (black) and in 100 ng of *Z*4-11Al *vs* 100 µl of EtOH (grey, brown and green) assays. Bars indicate Italian line (Black and grey), *Orco3* line (brown) and WT3 line (green). B) GC-EAD tracings showing responses of *D. suzukii* Italian, WT3 and *Orco3* to a blend (at 0.1 µg) of *Z*4-11Al and 2-phenylethanol (a positive control). Numbers in bracket indicate replicates; Asterisks indicate significant difference (p<*** 0.001; ** p<0.01; *p<0.05). C) The percentage of mated *D. suzukii* females that landed after an upwind flight in response to *H. uvarum* odour in a flight assay. Black and gray bars indicate landing when EtOH (10 µL/min) or *Z*4-11Al (10 ng/min dissolved in 10 µL EtOH), respectively, was sprayed into the plume of *H. uvarum* odours (n=73). Modified from paper IV.

That *D. suzukii* exhibits oviposition aversion in the presence of *D. melanogaster* associated chemical cues, lend support to previous studies (Shaw *et al.*, 2018; Dancau *et al.*, 2017). Both male and female exposed berries induced aversion, but female exposed berries exhibited stronger aversion than male exposed berries. We established that a female specific pheromone, *Z*4-11Al, is at least partly responsible for the aversion, and that *Z*4-11Al also antagonizes attraction of *D. suzukii* to *H. uvarum*. Furthermore, we show that the aversion in *D. suzukii* is mediated through olfaction, and knock-out of the odorant co- receptor disrupts the avoidance and response to *Z*4-11Al in *D. suzukii*. Nevertheless, we consider that additional or alternative compounds, such as cues emitted by microbes that are associated with *D. melanogaster* could trigger avoidance, too (Rombaut *et al.*, 2021; Sato *et al.*, 2021).

Previously, *Z*4-11Al was shown to antagonize courtship and attraction to food odours in *D. simulans* (Borrero-Echeverry *et al.*, 2022; Lebreton *et al.*, 2017). Our results confirm that the *D. melanogaster* pheromone, *Z*4-11Al, is perceived by heterospecifics, and adds to the list of contexts under which *Z*4-11Al has cross-species effect. While the ecological implication of *D. melanogaster– D. suzukii* interaction is not straightforward, we speculate that *D. suzukii* avoids competition with *D. melanogaster*. Such a strategy would benefit *D. suzukii* off-spring since they have longer developmental time (personal observation) and are competitively inferior relative to *D. melanogaster* off-spring (Dancau *et al.*, 2017). Exploitation of environmentally sound chemicals, such as heterospecific cues, could be of practical relevance towards management of the invasive pest, *D. suzukii.*

6. Conclusion and future perspectives

When ancestors of the cosmopolitan *D. melanogaster* spread “Out of Africa,” it would have been a long shot, to hypothesize that a species, *D. suzukii*, will emerge in the lineage and move “Into Africa” as an invasive pest. In both species, human activities have been critical in their dispersal and establishment. Moreover, the sense of smell, has been behind their success. Based on odour-mediation as a theme, this thesis had basic and applied aims.

From the basic aspect, I have shown the ability of human to detect and discriminate commensal *D. melanogaster* based on the female pheromone (Paper I). It should be of interest to know what constitutes human odour space with reference to the evolutionary cradle of *D. melanogaster*. Perhaps through this an inference can be made as to why there is a convergence of perception for the fly pheromone. By focusing on reproductive isolation in the genus *Drosophila* (Paper V), I have discussed that the role of aldehydes produced during spontaneous oxidation of cuticular dienes might have been overlooked. To what extend abiotic and biotic factors are involved in the oxidation remains to be investigated. Ascertaining the role of the aldehydes, olfactory receptors behind their perception and evolutionary relationship with the *D. melanogaster* Or69a, could shed light on whether evolutionary necessity, if any, can account for their existence and functionality.

For *D. suzukii*, I have shown that as simple as a home-made trap could be (Paper II), the informational output enhances our basic understanding of distribution and ecology of the invasive pest. Thus, the findings could be used for decision making, particularly to inform an area-wide monitoring program in areas with limited data on *D. suzukii*. Just like the old regions that play host to *D. suzukii*, the new regions would require effective trapping and management approaches. Therefore, improving the selectivity of the existing trapping baits is a necessity. My thesis has hinted at achieving the selectivity by applying the knowledge of *D. suzukii*- *H. uvarum* association (Paper III). To realize the full potential of the association, the *H. uvarum* headspace-based blend should be optimized. With *D. suzukii* emerging as a model organism for comparative studies, a complete picture of the ecological interaction between *D. suzukii* and *D. melanogaster*, remains to be painted. Meanwhile, I have shown that a female specific signal could partly be responsible for such an interaction (Paper IV). Further testing would be necessary to establish the feasibility, efficacy, and selectivity of the pheromone as an add-on management tool against *D. suzukii.*

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Popular science summary

Humans and the common fruit fly, *Drosophila melanogaster*, are two separate worlds apart, but at times, looks can be deceiving! They historically originate from Sub-Saharan Africa, both spread and share different corners of the world, and both uses their “nose” to gratify their preference for food, find partners and to detect danger. Because of the spread, you often find in gardens and kitchens, the cosmopolitan group of the flies. But part of the group, flies of the so-called *D. melanogaster* Zimbabwe strains, still exist in the wild areas of origin. While working with the common fly in the lab, we noticed that we smell female cosmopolitan flies and that they smell differently compared to conspecific Zimbabwe females. Perhaps you are asking how and why! We found that females of the two strains produce different chemical cues and that we are sensitive to the cosmopolitan female pheromone. Through a sniff, we can smell the difference. A straightforward explanation as to why we are sensitive to the fly pheromone is to be established.

In Japan, another fly, the spotted wing drosophila (SWD), *Drosophila suzukii*, closely related to the common fruit fly was reported in 1939. Unlike the common fly, SWD infests and damages soft fruits causing economic losses in Asia, and nowadays also in the Americas, and Europe where it became an invasive pest. In Sub-Saharan Africa, it was unknown if SWD was present. We found that it is present in Kenya. Currently, vinegar-wine based traps are used worldwide to monitor SWD, but the traps catch other nontargeted insects, too. We found that we can use volatiles of *Hanseniaspora uvarum,* which is a yeast associated with SWD, to enhance trap selectivity for SWD. To apply our results, the volatile components and their ratio need to be optimized. In addition, we found that the female pheromone *Z*4-11Al of *D. melanogaster*, can prevent SWD from laying eggs on berries. We recommend doing field testing to find out if and how farmers can use the pheromone in keeping away SWD from fruit orchards while minimizing financial losses and application of pesticide.

Populärvetenskaplig sammanfattning

Människan och bananflugan, *Drosophila melanogaster*, kan tyckas vara från två helt skilda världar, men ibland kan skenet bedra! Båda har ett gemensamt ursprung i subsahariska Afrika och har därifrån spridit sig till olika hörn av världen. Båda använder också ”näsor”, dvs doftorgan, för att kontrollera sina val av mat och för att upptäcka faror (bananflugan använder dofter också för val av partner). Den grupp av bananflugor som spridits över världen och som du kan hitta i ditt kök eller din trädgård kallas kosmopolitisk. Men i de afrikanska ursprungsområdena finns fortfarande den originalgrupp kvar som kallas Zimbabwe. När vi arbetade med de två olika grupperna av bananflugor i laboratoriet upptäckte vi att det är möjligt att känna skillnad på lukten av kosmopolitiska honor och honor från Zimbabwegruppen. Hur är detta möjligt? Vi upptäckte att honorna i de två grupperna producerar olika substanser som de frisläpper som kemiska signaler för att locka till sig hanar (sk. feromoner) och att vår näsa är speciellt känslig för feromonet från honor i den kosmopolitiska gruppen. Med en enda sniffning kan vi känna skillnaden! Vi söker nu vidare efter förklaringen till varför våra näsor är så känsliga för ett bananflugeferomon.

I Japan rapporterades 1939 en nära släkting till bananflugan, den sk. körsbärsättiksflugan, *Drosophila suzukii.* Till skillnad mot bananflugan är körsbärsättiksflugan en fruktad skadeinsekt på mjuka frukter och bär som orsakar enorma ekonomiska förluster i Asien och numera också i Amerika och Europa där den blivit en invasiv art med snabb spridning. Hittills har det inte varit känt om den också förekommer i subsahariska Afrika, men vi har nu för första gången hittat arten i Kenya. Traditionellt har körsbärsättiksflugan övervakats med vinägerfällor, men dessa är inte specifika utan fångar många andra arter också. Vi upptäckte att genom att tillsätta doftämnen från svampen *Hanseniaspora uvarum* till fällorna lyckades vi förbättra träffsäkerheten och minska antalet bifångster. Arbete att ytterligare optimera sammansättningen av de aktiva, flyktiga svampdofterna fortsätter. Vi har också upptäckt att feromonet från bananflugan hindrar körsbärsättiksflugan från att lägga ägg på bär. Vi rekommenderar nu utvidgade fälttester för att avgöra om och hur odlare kan använda feromoner i effektiva metoder för att hålla körsbärsättiksflugan borta från frukt- och bärodlingar och därigenom minimera både ekonomiska förluster och behovet av kemiska bekämpningsmedel.

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Appendix

Through part of the studies carried out in Paper IV, we were granted a patent, SE544112 C2, registered by the Swedish Patent and Registration Office.

Patentee: Paul Becher and **Charles Kwadha**

Title: Deterrent composition and method

Abstract

A method of deterring *Drosophila suzukii*, comprising providing an effective dose of *cis*-4-undecenal, or a precursor yielding *cis*-4-undecenal through autoxidation, at a location from which *Drosophila suzukii* should be deterred, is disclosed. Use of *cis*-4-undecenal for deterring *Drosophila suzukii*, and a deterrent composition and a device for deterring *Drosophila suzukii*. The deterrent composition and device comprising *cis*-4-undecenal or a precursor yielding *cis*-4-undecenal through autoxidatlon, are also disclosed.

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RAPID COMMUNICATION

Detection of the spotted wing drosophila, *Drosophila suzukii*, in continental sub-Saharan Africa

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Abstract

The spotted wing drosophila, *Drosophila suzukii* Matsumura, is an insect pest of soft-skinned fruit, native to Eastern Asia. Since 2008, a world-wide dispersal of *D. suzukii* is seen, characterized by the establishment of the pest in many Asian, American and European countries. While the potential for invasion of continental Africa by *D. suzukii* has been predicted, its presence has only been shown for Morocco in Northern Africa. Knowledge about a possible establishment in other parts of the continent is needed as a basis for pest management. In 2019, we carried out a first survey in three counties in Kenya to monitor for the presence of *D. suzukii* using traps baited with a blend of apple cider vinegar and red wine. A total of 389 *D. suzukii* flies were captured in a fruit farm at Nakuru county, with more female flies being trapped than males. We confirmed the morphological identification of *D. suzukii* using DNA barcoding. In 2020, we performed a follow-up survey at 14 locations in six counties to delimit the distribution of *D. suzukii* in the main berry growing zones in Kenya. The survey indicated that so far *D. suzukii* is restricted to Nakuru county where it was initially detected. This is the first study to provide empirical evidence of *D. suzukii* in continental sub-Saharan Africa, confirming that the pest is expanding its geographic range intercontinentally. Given the high dispersal potential of *D. suzukii*, a concerted effort to develop management strategies is a necessity for containment of the pest.

Keywords *Drosophilidae* · Fruit fly · Global · Invasive pest · Soft fruits ·

Key message

- Despite the global spread of *Drosophila suzukii*, its distribution in Africa lacks clarity.
- We carried out a monitoring study in Kenya by using traps and investigating fruit samples.
- *Drosophila suzukii* was captured in raspberry, strawberry blueberry and pomegranate plants.
- Morphological identification was confirmed using DNA barcoding.
- The findings confirm the presence of *D. suzukii* in continental sub-Saharan Africa.

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Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01330-1.

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Introduction

Native to Asia, the spotted wing drosophila (SWD) *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) was first reported as a pest in wild blueberries and cherry orchards in Shioyama Town, Japan, in 1916 (Kanzawa 1939). Since then, *D. suzukii* has become known as a pest of soft and stone fruits which inflicts serious economic loss to the fruit industry (Asplen et al. 2015). The loss is attributed to the serrated ovipositor of the female with which it pierces the skin of ripening fruits. In addition to the direct damage through oviposition and subsequent larval feeding, damaged fruits also get exposed to secondary infestation by other drosophilids and opportunistic pathogens (Rombaut et al. 2017).

Drosophila suzukii has nowadays been reported in many parts of the globe outside Asia. In 1980, it was reported in Hawaii and later in 2008 in the Pacific coast of California as an invasive pest (Bolda et al. 2010; Goodhue et al. 2011). Subsequently, *D. suzukii* has been found in other parts of the Americas (Hauser 2011; Walsh et al. 2011) as well as across European countries (Calabria et al. 2012; Ørsted and Ørsted 2019). Comparisons between selected European and North American *D. suzukii* populations reveal that invasions occurred through different routes as the latter populations were suggested to originate from southeast China while the former were traced to northeast China (Rota-Stabelli et al. 2020). Overall, *D. suzukii* shows rapid and ongoing inter-and intracontinental dispersal. Recently, *D. suzukii* was confirmed from samples collected in Morocco during 2017 and 2018, demonstrating presence in Northern Africa (Boughdad et al. 2020). Observations of *D. suzukii* are reported in Réunion island (IPPC 2017) and in the Comoros archipelago (Hassani et al. 2020). However, it was unknown if *D. suzukii* is present in continental sub-Saharan Africa.

Distribution models predict the establishment of *D. suzukii* in numerous sub-Saharan countries (Dos Santos et al. 2017) which would likely affect fruit production similar as seen in other regions. In sub-Saharan Africa, fruits generally are produced through irrigated, urban-based or highland mixed-rainfed farming systems (Costa et al. 2013; Dixon et al. 2001). While irrigated and urban-based farming systems mainly target foreign markets, rain-fed farming systems mostly supply local markets (Costa et al. 2013; Dixon et al. 2001). Moreover, unlike rain-fed farming systems which are characterized by indigenous skills and knowledge, irrigated and urban-based farming systems apply modern farming technologies, including integrated pest management (De Bon et al. 2014; Pretty and Bharucha 2015).

Generally, the global spread of *D. suzukii* is aided by international trade (Cini et al. 2014). Sub-Saharan Africa is trading commercial commodities such as fruits and vegetables, on the international market (European Union 2019; Legge et al. 2006). For example, South Africa, Cameroon, Kenya, Ghana and Ethiopia are exporting off-season and tropical fruits to both regional and international trade partners (Hoffmann and Vossenaar 2008).

Kenya, as a strategically located country, provides a bridge, through its port of Mombasa, that allows for cross-border movement of commodities to the neighboring landlocked countries including Uganda, Burundi, Rwanda and South Sudan (Sebuny 2015). Consequently, Kenya and the neighboring partner countries are exposed to a high risk of invasion by economically significant insect pests. A prime example of such an invasion is represented by *Bactrocera* species, such as *Bactrocera dorsalis* (Hendel) (Diptera; Tephritidae), which originates from Asia and was first recorded in Kenya with subsequent reports of its establishment in other Eastern, Central and Southern African countries (Drew et al. 2005; Lux et al. 2003). As of 2018, Kenya was importing strawberries, raspberries, blackberries, gooseberries, cherries and plums, which all are known as *D. suzukii* host fruits (International Trade Centre 2019). Of the fruit-importing countries, South Africa, Italy, Egypt, Spain, Chile and India the four non-African countries have confirmed the presence of *D. suzukii* (International Trade Centre 2019; CABI 2019; Kenis et al. 2016). Introduction of *D. suzukii* might lead to a permanent invasion and dispersal of the pest in sub-Saharan Africa. Through inductive modeling, Dos Santos et al. (2017) previously showed that southwestern Kenya and bordering countries (Uganda and Tanzania) have suitable abiotic conditions that would support *D. suzukii* establishment.

Evidently, the above-mentioned factors provided a backdrop on which we hypothesized that *D. suzukii* might already have been introduced into continental sub-Saharan Africa and be present in soft and stone fruit cultivating regions. To address this hypothesis, we carried out a monitoring survey during 2019 and 2020. We report the detection but apparently restricted distribution of *D. suzukii* in Kenya. Species identity was confirmed using morphological features together with DNA barcoding of the mitochondrial cytochrome oxidase subunit I (*COI*).

Material and Methods

Detection survey in 2019

To monitor for potential invasion of *D. suzukii* in Kenya, a survey was carried out during the dry season, from 13th to July 20th, 2019, in three counties; Nairobi county (farm: Duduville campus of the International Centre of Insect Physiology and Ecology (*icipe*), 1° 22′S, 36° 89′E, about

1, 600 m above sea level, hereafter masl); Kiambu county (farm: Sasini estate 1° 16′S, 36° 89′E, about 1,700 masl) and Nakuru county (farm: Longonot farm 0° 83′S, 36° 38′E, about 1,935 masl). We used the Leaflet package in R v 2.0.2 (Cheng et al. 2018) to show the locations on a map (Fig. S1). Brief descriptions of *icipe,* Sasini estate and Longonot farm are given in the supplementary material. In each of the farm, 10 traps (1 trap/site, see supplementary for details; Fig. S2) of 60-mL volume filled with 40 mL of a blend of apple cider vinegar and red wine (20:80 v:v) were used (Huang et al. 2017; Landolt et al. 2012). At *icipe* and Sasini, we monitored crops cultivated in a mixed-cropping system. At Longonot, we sampled raspberries (*Rubus idaeus*), strawberries (*Fragaria ananassa*), blueberries (*Vaccinium corymbosum*) and pomegranates (*Punica grantum*) (Table S1) grown in separate tunnels (Fig. S3). Two traps were placed per tunnel. At the time of the survey, fruit ripening was more advanced in raspberries than in the other crops. After a week, the captured insects were collected and inspected for the presence of *D. suzukii* by morphological features using a Zeiss microscope (Göttingen, Germany) fitted with an Axiocam 105 color microscope camera (Göttingen, Germany). Generally, the adults were about 2–3 mm long, having red eyes with brown thorax and abdomen, with unbroken bands. The males were characterized by a dark spot on each wing (Fig. S4a) and a sex comb on the first and second segment of the foreleg with 3–6 teeth running parallel to the feet (Fig. S4b). Females were identified based on the distinctive ovipositor with a pronounced serration that is different from *D. subpulchrella* (Atallah et al. 2014; Vlach 2010) (Fig. S4c).Through a weather information provider (Visual Crossing 2020), daily data on temperature, relative humidity and precipitation were extracted from nearest accessible weather stations in Nairobi, Kiambu and Naivasha (Nakuru) representing the respective survey sites (Table S3). In a follow-up survey in 2020 (see below), weather data were recorded in Laikipia, Murang'a, Nyandarua and Nyeri (Table S4).

Follow-up survey in 2020

As it was unclear whether or not the pest had established and dispersed since its detection in Longonot in 2019, we carried out a follow-up survey between May 20 and June 11, 2020. A total of 11 farms and 3 roadsides distributed in major berry growing areas including Longonot farm (Nakuru county), Kiambu, Nyandarua, Murang'a, Nyeri and Laikipia counties were surveyed (Fig. S1 and Table S2). Roadsides have earlier been shown as suitable sites to detect *D. suzukii* (Lengyel et al. 2015). Baited Drososan traps (Koppert Biological Systems, the Netherlands) were set up at 25 sites (one trap per site) at the 14 locations (Table S2). Fruits monitored at the farms were cultivated in tunnels. After 5–7 days, traps were collected for sorting and identification at i*cipe*. The number of caught flies was computed as daily catches of flies per trap. Like in the 2019 survey, traps were set up and collected once.

Samples of *D. suzukii* from both the 2019 and 2020 surveys are stored in 70% ethanol at *icipe* and are accessible for future reference, through the code SWD-Longonot-07-2019.

Survey on infestation of berries by *D. suzukii*

To assess host infestation, we sampled ripe raspberries, strawberries, blackberries (*Rubus fruticosus*) and blueberries grown at 11 farms at different locations. The specific types of fruit grown and investigated at different farms are provided in Table S2. For each type of fruit investigated at a respective farm, at least 0.2 kg of berries was sampled. Each sample consisted of berries that were handpicked from the plants and berries that had fallen to the ground. With the exception of *R. idaeus* and *V. corymbosum* at Longonot, which were collected at two dates, fruits at other farms were collected once. Collected berries were kept separately by type in 2-L plastic containers (Kenpoly Manufacturers Ltd, Nairobi, Kenya), covered with lids fitted with fine netting material. Samples were transported to the laboratories at the Animal Rearing and Containment facility at *icipe*. Sample weight was measured using an electrical balance (KERN PCB 2500, KERN & Sohn GmbH Ziegelei Balingen, Germany). Developing larvae were protected from drowning in excess juice by lining each container with four layers of Velvex premium kitchen towels (Chandaria Industries Ltd, Nairobi, Kenya) which were exchanged twice a day.

Pupae were collected, transferred into a Petri-dish lined with moistened paper towel and kept in a Perspex cage ($0.2 \times 0.2 \times 0.2$ m) fitted with a fine mesh sleeve. Emerging adults of *D. suzukii* were counted and recorded as flies per kilogram of fruit.

Molecular identification

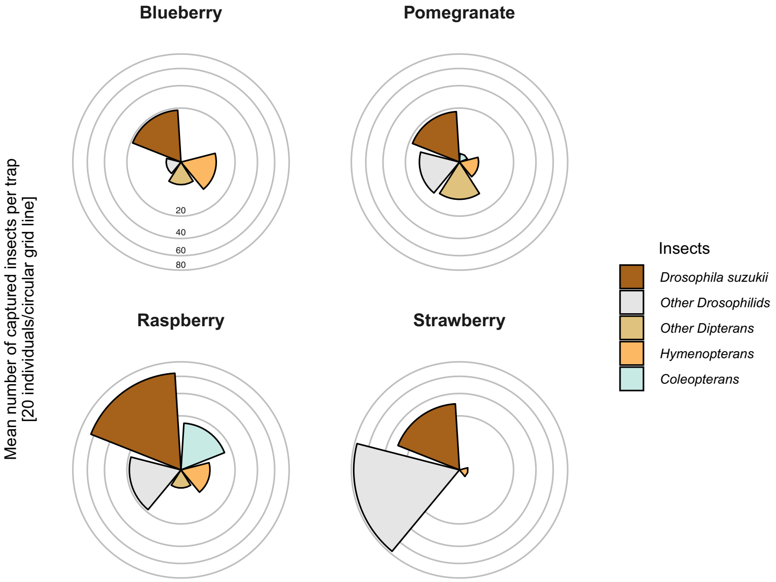
We extracted the genomic DNA from eight flies ($n = 4$ per sex) caught in 2019 using phenol-chlorofom (Sambrook and Russell 2006) and from three flies (2 males and 1 female) that emerged from incubated berries in 2020 using Isolate II Genomic DNA kit. For polymerase chain reaction (PCR) amplification, we used *D. suzukii* specific primers to amplify the barcode region of the cytochrome oxidase I (*COI*) for 2019 samples and general primers (Hajibabaei et al. 2006) for 2020 samples. Amplified fragments were purified and bidirectionally sequenced at Macrogen Europe BV (Meibergreef, Amsterdam, the Netherlands). Following visual inspection and manual trimming of the DNA sequence electropherograms, we aligned forward and reverse sequences using ClustalW algorithm (Bioedit v. 7.2.5) (Hall 1999). The representative sequences were aligned to reference sequences from the National Center for Biotechnology Information (NCBI). Additionally, we compared the sequences with those of closely related species and generated a maximum likelihood phylogenetic tree (Fig. S5) with 1000 bootstraps using MEGA X v 10.0.5 (Kumar et al. 2018). To generate the tree, we aligned the sequences using the default settings of MUSCLE algorithm in MEGA X. The resultant alignment was used to predict the best model for constructing a phylogenetic tree using Tamura–Nei model (Tamura and Nei 1993) which had the lowest Bayesian Information Criterion (BIC = 4561.79). All the sequences submitted to GenBank can be accessed through: accession MN689051-MN689058 for 2019 samples, and accession MT966718, MT975699 and MT981339 for 2020 samples. More details are provided in the supplementary material.

Results

Out of the 30 sites at the three locations monitored in the 2019 survey, *D. suzukii* was detected at ten sites at Longonot farm, Nakuru county. Flies were first identified by morphological features. We confirmed the morphological identification of *D. suzukii* through *COI* DNA fragment amplification from genomic DNA, followed by direct sequencing and BLAST searches. The resulting sequences (Table S5a) were aligned to reference sequences from NCBI compared with publicly available data on GenBank and yielded a similarity score of 100% with *D. suzukii* samples from Switzerland (Accession number MG605095.1), Turkey (Accession number MK435596.1) and Italy (Accession number KJ671599.1) (Kuyulu et al. 2019) (Fig. S5). A total of 389 *D. suzukii* flies were captured in the 7-day period of the survey, in which females accounted for 62.0% (95% confidence interval of 56.9–66.8%, for binomial distribution) of the total catches. In detail, with the two traps per plot, 130 and 127 *D. suzukii* flies (80 and 78 females, respectively) were trapped in the two raspberry plots, 60 flies (47 females) in the strawberry plot, 37 flies (15 females) in the blueberry plot and 35 flies (21 females) in the pomegranate plot (Fig. 1). Other species of Diptera, Coleoptera and Hymenoptera were captured at Longonot, Sasini and *icipe;* these specimens were stored but not further identified. In Longonot, *D. suzukii* represented 60.8% of all the captured *drosophilids.*

In the follow-up survey in 2020, *D. suzukii* was detected in Nakuru county but only at Longonot farm where we had trapped flies already in 2019. From the monitoring traps, 358 *D. suzukii* flies were captured, in which 257, 63 and 38 flies were caught in raspberries, blueberries and strawberries, respectively. Moreover, 799 adults of *D. suzukii* emerged from incubated fruit, of which raspberries had the highest number of flies (782, compared to 14 and 3 flies that emerged from blueberries and strawberries, respectively). *Drosophila suzukii* was neither observed in traps nor emerged from incubated fruits collected at berry growing areas in Kiambu, Nyandarua, Murang'a, Nyeri and Laikipia counties. We further confirmed the identity of *D. suzukii* captured in the 2020 survey through morphological and molecular

Fig. 1 *Drosophila suzukii* monitoring results from a fruit farm in Kenya. Radial plots illustrate the mean number of *D. suzukii,* other drosophilids, other Dipterans, Hymenopterans and Coleopterans per trap caught across four different fruit crops in 2019. The circular grid lines of each radial plot represent 20 captured individuals belonging to different insect categories listed above



identification. The resulting sequences (Table S5b) gave a similarity score of 94–100% with reference sequences in GenBank (MK435596.1 and MK801757.1).

Discussion

In the last decade, *D. suzukii* has spread from its native region in Asia to America and Europe, and the possibility for further dispersal to continental Africa was predicted (Dos Santos et al. 2017). Recently, the presence of *D. suzukii* was reported in Morocco, North Africa (Boughdad et al. 2020). Through monitoring, we show the presence but apparently restricted distribution of *D. suzukii* in Kenya, sub-Saharan Africa. Morphological examination and molecular confirmation by *COI* barcoding were used to prove identification at the species level.

During 2019, we captured *D. suzukii* in Longonot, a commercial farm growing blueberries, strawberries, raspberries and pomegranates, fruits which are known to support the development of the flies (Arnó et al. 2016; Wang et al. 2019). *Drosophila suzukii* was captured in plots of all different fruits. The establishment of invasive *D. suzukii* at Longonot farm was confirmed in 2020 where the pest was found both in monitoring traps and sampled fruit. Traps in raspberry tunnels caught most *D. suzukii* in 2019 and raspberries were more infested than blueberries and strawberries in 2020. This suggests that *D. suzukii* infestation differs across fruits and that raspberry is the most susceptible crop to *D. suzukii* at Longonot farm. Oviposition and infestation are known to differ among host species with raspberries representing one of the preferred fruits (Burrack et al. 2013; Lee et al. 2011). Moreover, more ripe berries were observed in plots with raspberries compared to plots with other types of fruits. *Drosophila suzukii* prefers ripe to unripe berries (Karageorgi et al. 2017; Keesey et al. 2015) and captures of *D. suzukii* across orchards in close proximity are known to fluctuate depending on fruit phenology (Lee et al. 2015; Wang et al. 2016). In the 2019 survey, we did not investigate the infestation of fruit. However, damage by unspecified insect pests was occurring in the farm as was reported by the field manager. The detection of *D. suzukii* since 2019 and the emergence of the pest from incubated fruits collected during the 2020 survey provide a likely explanation for the reported insect damage. At Longonot, the mixed production of different crops with successive availability of ripe fruits throughout the season, as well as the practice of drip irrigation and cultivation in tunnels, might provide most suitable microhabitats facilitating *D. suzukii* establishment at the farm (Diepenbrock and Burrack 2017; Khaliq et al. 2014; Toxopeus et al. 2016).

Despite the availability of host fruits at the farms that represent 11 out of the 14 locations surveyed in 2020, *D. suzukii* was only found at Longonot farm. This, in consequence, does not allow conclusions about the presence or absence of the pest apart from Longonot. Repetitive detection of *D. suzukii* at Longonot proves the validity of our monitoring technique and suggests that the fly would have been detected if present. *Drosophila suzukii* has been shown to disperse over long distances (Tait et al. 2018). The establishment at Longonot implies the possibility of an area-wide dispersal if *D. suzukii* is not contained. In the recent past, invasive pest species of the genus *Bactrocera* have caused substantial losses to Africa's fruit industry (Drew et al. 2005; Ekesi et al. 2016; Lux et al. 2003). In Europe and America, *D. suzukii* infestation has caused huge yield losses and costs exceeding $100 million per fruit per year (De Ros et al. 2013; Mazzi et al. 2017; Walsh et al. 2011), and therefore, Kenya and other African countries are likely to experience similar economic impact by the newly found pest. There has been a growing interest amongst small-scale farmers in Kenya, to grow soft fruits such as plums (Mbora et al. 2008) and berries. In the neighboring country, Ethiopia, strawberry cultivation is on the rise with small- and medium-scale growers adopting the practice. The detection and establishment of *D. suzukii* are a threat to the potential expansion of berry cultivation and trade in the region. Therefore, our findings serve a two-front wake-up call to stakeholders of the Kenyan and regional fruit industry. It calls for continuous region-wide monitoring to establish the extent of *D. suzukii* distribution, from which management programs can be defined and executed (Kenis et al. 2016).

In addition to monitoring, other measures have been proved as important tools to control *D. suzukii* and should support the development of integrated management practices (IPM) in Kenya and other African countries. Informing growers about the appearance of *D. suzukii* and how to recognize specific damage and the pest itself is a most urgent task. Self-made traps similar to that applied in our study (Fig. S2) could facilitate on-farm monitoring. Directed sanitary measures on farms should be implemented to reduce development of *D. suzukii*, e.g., in decaying fruit left on the plant or fallen to the ground (Haye et al. 2016). Further, adjustments in cultivation practice such as selection of less susceptible varieties or harvesting at an early stage of ripening before berries become soft and more likely to be infested should be considered for the coming growing seasons (Hampton et al. 2014; Lee et al. 2016). Another measure that could be applicable to farmers is the use of netting to reduce the entering of *D. suzukii* into tunnels or to cover and protect crops such as blueberries (Del Fava et al. 2017). Insecticides have been intensively studied and are applied in conventional and organic production (Van Timmeren and Isaacs 2013). For example, spinosad is one of the widely used insecticides for control of *D. suzukii* and even used in organic production (Bruck et al., 2011).

Recent studies increase the general concern about insecticide resistance in *D. suzukii* advocating the application of sustainable pest control methods (Gress and Zalom 2019). Biological control is a promising route to reduce *D. suzukii* infestation using natural enemies and insect pathogens (Becher et al. 2018; Biondi et al 2020; Daane et al. 2016; Gabarra et al. 2015; Lee et al. 2019; Wolf et al. 2020). Findings on the specificity of parasitoids with respect to *Drosophila* host species, different fruit species as well as fruit ripeness give grounds for hope toward the development of biological control (Girod et al 2018; Wolf et al. 2020; Biondi et al 2020; Seehausen et al 2020). In our trap captures, non-targeted Coleopterans and Hymenopterans were recorded but not identified. Previously, predatory Coleopterans and parasitic Hymenopterans were reported in *D. suzukii* traps and monitoring (Wang et al. 2016; Wolf et al. 2018). The possible existence of natural enemies in the area warrants follow-up studies. Generally, *D. suzukii* is not limited to sites of fruit production but is often found in forests or unmanaged areas which emphasizes the need for area-wide and long-term control strategies exploiting approaches such as biological control or the sterile insect technique (Haye et al. 2016; Seehausen et al. 2020; Nikolouli et al. 2020).

The detection of *D. suzukii* at only one location indicates that the distribution of *D. suzukii* in Kenya still is restricted and the invasion is at an early stage. New pest invasions are characterized by an arrival and an establishment phase that generally allow management to interfere with pest dispersal (Kolar and Lodge 2001; Liebhold and Tobin 2008). Overall, the combination of different measures such as monitoring, chemical, biological and cultural management, combined with preventive cultural practice, provides a toolbox to develop a sustainable IPM approach at local and landscape scale (Cini et al. 2012; Del Fava et al. 2017; Haye et al. 2016), targeting *D. suzukii* as a new pest in sub-Saharan Africa.

Dos Santos et al. (2017) had predicted that Tanzania offers a larger region suitable for *D. suzukii* establishment than Kenya, suggesting that while this is the first record of the pest in continental sub-Saharan Africa, a region-wide monitoring program is required to obtain a better overview on the current status of *D. suzukii* occurrence. Previous studies established multiple invasions in other infested countries (Carvajal and Markow 2010; De la Vega et al. 2020; Lavrinienko et al. 2017). It would be intriguing to study population genetics of the Kenyan population and establish the possible nature of the invasion. Clearly, more information is needed about the distribution and phenology of *D. suzukii* in Africa, potential host fruits on farms and in the wild, and the occurrence of natural enemies. Cost estimations on the economic impact and control programs in Kenya and other African countries would be helpful to guide future strategies for adequate management. Altogether, understanding the population dynamics of *D. suzukii* in the local ecological, environmental, landscape and horticultural context will provide a benchmark for successful management.

In conclusion, by using traps we showed the presence of *D. suzukii* at Longonot farm (Nakuru county) in Kenya. Through host incubation in the laboratory, we confirmed infestation of the berries collected from the farm. We have through morphological examination and DNA barcoding confirmed the identity of the collected flies. Altogether, *D. suzukii* is present in Kenya but was only detected in one out of six monitored counties suggesting that the pest is at an early phase of invasion and might be absent in the main host-fruit production areas of Kenya. Our findings add to the growing list of countries recently invaded by *D. suzukii*, illustrating the exceptionally fast and ongoing geographical range expansion of this pest insect. *Drosophila suzukii* has a high potential of dispersal, which necessitates quick and concerted efforts to prevent further distribution in the region.

Author contribution

CAK, IK, GR and PGB conceived the idea. CAK, IK, GR, SN, MK, SAM, PN and PGB designed the experiments. CAK, SN, SAM, PWN, MK, MKG and GGK conducted the survey. CAK and SN did morphological identification. LAO, FMK, SR and HMGL did molecular identification and analysis. MK assayed fruit incubation. CAK and GR analyzed the trap capture results. CAK and PGB wrote the manuscript with contributions by SR, SN, SAM and FMK. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This study did not involve any human or animal (vertebrate) subject.

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Special Collection: Research Advances in Spotted-Wing Drosophila suzukii Management

Hanseniaspora uvarum Attracts *Drosophila suzukii* (Diptera: Drosophilidae) With High Specificity

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Abstract

Since the early phase of the intercontinental dispersal of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), fermentation baits have been used for monitoring. Self-made lures and commercial products are often based on wine and vinegar. From an ecological perspective, the formulation of these baits is expected to target especially vinegar flies associated with overripe fruit, such as *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae). *Hanseniaspora uvarum* (Niehaus) (Ascomycota: Saccharomyceta) is a yeast closely associated with *D. suzukii* and fruit, and furthermore attractive to the flies. Based on this relation, *H. uvarum* might represent a suitable substrate for the development of lures that are more specific than vinegar and wine. In the field, we therefore, compared *H. uvarum* to a commercial bait that was based on vinegar and wine with respect to the number of trapped *D. suzukii* relative to other drosophilids and arthropods. Trap captures were higher with the commercial bait but specificity for *D. suzukii* was greater with *H. uvarum*. Moreover, *H. uvarum* headspace extracts, as well as a synthetic blend of *H. uvarum* volatiles, were assayed for attraction of *D suzukii* in a wind tunnel and in the field. Headspace extracts and the synthetic blend induced strong upwind flight in the wind tunnel and confirmed attraction to *H. uvarum* volatiles. Furthermore, baited with *H. uvarum* headspace extract and a drowning solution of aqueous acetic acid and ethanol, 74% of field captured arthropods were *D. suzukii*. Our findings suggest that synthetic yeast headspace formulations might advance the development of more selective monitoring traps with reduced by-catch.

Key words: horticultural pest, invasive pest, pest surveillance, semiochemical, spotted wing drosophila

Traps baited with attractant lures are a basic and most widely used tool for insect management. The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a worldwide spreading pest, and trap lures are substantially required for detection and monitoring occurrence and dispersal (Calabria et al. 2012, Walsh et al. 2011, Dalton et al. 2011, Pelton et al. 2016, Kwadha et al. 2021), seasonal population development (Hamby et al. 2014), management decisions (Cha et al. 2018a), finding natural enemies (Abram et al. 2020), and for population control by attract-and kill (Haye et al. 2016, Rice et al. 2017). Development of trap lures has recently been reviewed by Tait et al. (2021).

Due to the relevance and range of trapping applications, a substantial research effort has been made to optimize trap design (Lee et al. 2012, 2013; Renkema et al. 2014; Kirkpatrick et al. 2018) and chemical attractants (Landolt et al. 2012a; Cha et al. 2014, 2017; Kleiber et al. 2014; Frewin et al. 2017; Đurović et al. 2021; Larson et al. 2021).

The efficacy of insect traps and lures is determined by their attractant power as well as their specificity towards the target species (Wall 1990). Insect pheromones are highly species-specific and efficient at low release rates and therefore widely used for trapping of lepidopteran and coleopteran insects (Witzgall et al. 2010). However, pheromones have so far not been developed for long-range attraction and trapping of *Drosophila* flies (but see Lebreton et al. 2017).

Fermented fruit is known to attract *Drosophila* flies including *Drosophila melanogaster*, and fermentation products like vinegar and wine are therefore applied for fly trapping (Zhu et al. 2003, Becher et al. 2010, Birmingham et al. 2011). Not surprisingly, fermented fruit and vinegar were used also for trapping *D. suzukii*, leading to the development of commercial lures (Kanzawa 1939, Dreves et al. 2009, Landolt et al. 2012a, Cha et al. 2014). For example, one of the commercial traps used for monitoring and mass-trapping of *D. suzukii* is the disposable Riga trap (Riga AG, Ellikon a.d. Thur, Switzerland) which contains a vinegar-wine based bait (Haye et al. 2016). The Riga trap is often used as a reference for comparison with other attractants or traps (e.g. Tonina et al. 2018, Noble et al. 2019, Jones et al. 2021). Wine-vinegar blends were originally studied for the development of *D. suzukii* management by Landolt et al. which led to a monitoring bait based on a mixture of four individual wine and vinegar components forming the backbone of commercially available trap lures (Landolt et al. 2012a,b; Cha et al. 2013, 2015). Despite the wide use of current trap lures, lack of species-specificity has been cited as shortcoming (Cha et al. 2018a, Larson et al. 2021).

From an ecological point of view, vinegar and wine seemingly relate to vinegar flies that infest overripe fruit primarily, whereas *D. suzukii* typically infests fruit even before ripeness (Walsh et al. 2011). Hence, chemical cues more closely related to the ecology of *D. suzukii* could be a basis for developing more specific baits and trap lures (Cloonan et al. 2018).

The yeast *Hanseniaspora uvarum* (Niehaus) (Ascomycota: Saccharomyceta) is associated with *D. suzukii* and found in and on larvae, adult flies, and fruits (Hamby et al. 2012, Bellutti et al. 2018, Lewis et al. 2019). Moreover, previous bioassays demonstrated a strong attraction of *D. suzukii* to *H. uvarum* cultures (Scheidler et al. 2015, Mori et al. 2017, Rehermann et al. 2022). Furthermore, recent work demonstrated attraction of *D. suzukii* to *H. uvarum* in the field emphasizing the predictive value of laboratory studies (Jones et al. 2021). Nevertheless, the potential of *H. uvarum* to improve lure specificity remains to be investigated. We, therefore, compared *H. uvarum* to the Riga bait with respect to the number of trapped *D. suzukii* relative to other drosophilids. In the laboratory, we then tested *H. uvarum* headspace collections and a synthetic blend of selected headspace volatiles in a wind tunnel. Finally, we tested the potential of headspace and the synthetic blend of *H. uvarum* volatiles for *D. suzukii* field trapping.

Materials and Methods

Yeast Cultivation and Headspace Sampling

Colonies of *Hanseniaspora uvarum* (CBS 2570; Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands) grown on PDA (BD Difico, Potato Dextrose Agar: 39 g/L) were applied to prepare liquid precultures in PDB (BD Difico, Potato Dextrose Broth: 24 g/L). We used 1 ml of 1-d-old precultures as inoculum of 100 ml PDB to prepare fresh cultures for traps or sampling of headspace volatiles. Cultures (and precultures) were grown on a shaking incubator (25°C, 260 RPM) for 24 h and were in an exponential growth phase. Then, individual yeast cultures were transferred to 500-ml gas wash bottles for collection of the headspace volatiles. Using Teflon tubing, we connected each bottle with a micro gas pump (NMP830KNDC, KNF Neuberger, Inc, NY) that was pushing air through a charcoal air filter into the bottle. A Y-splitter at the gas outlet of the pump allowed to set the air flow (ca. 300 ml/min). The gas outlet of the bottle was connected to a Porapak air filter (Porapak Q, 80/100 mesh, Altech) for trapping the volatiles of the yeast, and further to the gas inlet of the pump. Volatile compounds were collected for four hours and then eluted from each filter with 300 µl ethanol.

Field Comparison of Riga bait and *H. uvarum* Culture

For all field experiments, we applied Red Drososan traps (Koppert Biological Systems), which are bucket traps that can be used with a liquid attractant in the bottom of the trap in which caught insects drown. In our first experiment, traps were baited with 80 ml fresh culture of *H. uvarum*, or with 80 ml bait transferred from Riga traps, respectively. The yeast cultures were moderately dense (optical density ca. 2.9 at 595 nm) and in exponential growth phase when transferred to the traps, i.e. the medium still contained sufficient resources for *H. uvarum* to survive and metabolize, while being expected to largely outcompete or suppress secondarily introduced microorganisms during the experimental time (Qin et al. 2017).

Traps were distributed on four dates (August 13, August 20, August 29, and September 5 in 2019) at 5 sites on the campus of SLU Alnarp (Sweden), mainly in the landscape laboratory, which is characterized by woodland, shrubs, and field edges, roadside plantations and waterbodies. A trap containing the Riga bait was paired with a trap containing yeast culture at distance of about 5 m. At the first two dates in August, we collected traps after 3 d while on the latter two dates, traps were collected after 4 d. Fruit trees near by the traps were recorded as dogwood (*Cornus* sp.), blackthorn (*Prunus spinosa*), guelder rose (*Viburnum* opulus), sea buckthorn (*Hippophae rhamnoides*), plums and mirabelle plums (*Prunus domestica*), hawthorn (*Crataegus* sp.), elderberry (*Sambucus nigra*), and morus (*Morus nigra*). Trapped specimen were counted and determined as SWD, other species of the Drosophilidae or other insect or arachnid species.

Wind Tunnel Tests

A wind tunnel equipped with a piezo electric sprayer was used to measure *D. suzukii* upwind flight attraction to *H. uvarum* headspace or a synthetic mix of headspace components (Becher et al. 2010). The piezo electric sprayer allows controlled vaporization of samples dissolved in organic solvents such as ethanol. The sprayer releases the vapor from a glass capillary horizontally introduced at the upwind end of the wind tunnel. The glass capillary is surrounded by a glass cylinder (60 x 95 mm diameter) which is covered with metal mesh (2 mm pore size) for protection of the set up. When sensing a highly attractive odor flies take off, fly upwind and try to approach the odor source which leads to contact with the metal mesh and most often landing on it.

Yeast headspace extracts were collected from fifteen 100-ml *H. uvarum* cultures cultivated for 24 h in PDB. The Porapak filter eluates of these collections were pooled together and stored in the freezer until used for wind tunnel tests. As highest concentration, we tested *H. uvarum* headspace extract at a concentration in which 1 min of spraying (in volumes of 10 µl/min) corresponded to 2 min of headspace sampling (n = 43 tested flies). In addition, we sprayed a 1:4 ($n = 56$) and a 1:8 ($n = 39$) ethanolic dilution of the extract.

Individual virgin, 3–6 d old, 24 h starved female *D. suzukii* flies from a laboratory rearing (fly stock originating from San Michele all'Adige, Italy) were released at the downwind end of the wind tunnel similar as described earlier (Mori et al. 2017). Fly behavior was observed for 3 min and events of "take-off and upwind flight" as well as "contact" with the metal mesh in front of the odor source was scored.

In addition, we tested fly attraction towards a synthetic blend of seven *H. uvarum* headspace volatiles, which were selected based on their electrophysiological activity on *D. suzukii* antennae (Cha et al. 2012, Revadi et al. 2015, Scheidler et al. 2015, Urbaneja-Bernat et al. 2021). Relative quantities were estimated from GC-MS measurements (6890 GC and 5975 MS; Agilent Technologies; splitless injection onto DB-wax (60 m x 0.25 mm i.d, 0.25 µm film thickness; J&W Scientific, Folsom, CA) with helium as mobile phase at 35 cm/s and a temperature program from 30°C to 225°C at 8°C/min, held for 3 min). Headspace components were tentatively identified based on their Kováts retention indices and mass spectra using the NIST reference library (Agilent), and standard reference compounds. Compounds were quantified based on their peak areas in relation to known quantities of injected reference compounds. The ethyl acetate peak of the ethanolic *H. uvarum* headspace extract, was covered by the ethanol solvent peak, and the ester was therefore quantified from a *H. uvarum* headspace sample that was eluted with hexane (data not shown).

The seven *H. uvarum* volatiles were blended in the following amounts based on the headspace analysis (ng; relative ratio in blend): acetoin (1.48; 7.4), sulcatone (0.02; 0.1), isoamyl acetate (1.16; 5.8), 2-phenylethanol (0.76; 3.8), phenylethyl acetate (0.94; 4.7), ethyl acetate (11.76; 58.8), and isoamyl alcohol (3.82; 19.1) resulting in a total of ca. 20 ng compound per µl ethanol for testing upwind flight attraction ($n = 40$). For getting an understanding of the threshold concentrations for behavioral activity we also tested dilutions of the blend containing 10 ng/µl ($n = 36$) and 1 ng/µl ($n = 40$) total compounds. Fly behavior, when exposed to ethanol, was measured to control the effect of the organic solvent, which was used for preparing the synthetic blends and headspace extracts ($n = 40$). All chemicals were obtained from Sigma Aldrich, but 2-phenylethanol from Merck.

Field Comparison of *H. uvarum* Headspace Extract, *H. uvarum*-Based Synthetic Blend and a Reference Blend

Based on the results from the wind tunnel assay, we performed a second field experiment at the SLU landscape laboratory, in which we compared catches from Drososan traps that emitted either collected *H. uvarum* headspace extracts or the synthetic blend of *H. uvarum* volatiles. In addition, we tested a synthetic reference blend that we formulated based on the study by (Cha et al. 2013), with modification as described below.

Yeast headspace was collected from six 100-ml cultures of *H. uvarum*, cultivated for 24 h in PDB as described above. The ethanolic filter eluates (300 µl per culture) of these six collections were pooled together and stored in a freezer until use for the field experiment.

The seven components of the synthetic *H. uvarum* blend were dissolved in ethanol in the amounts (µg) and relative ratios as follows: acetoin (7.4), sulcatone (0.1), isoamyl acetate (5.8), 2-phenylethanol (3.8), phenylethyl acetate (4.7), ethyl acetate (58.8), and isoamyl alcohol (19.1) resulting in a total of ca. 100 µg compound per µl ethanol.

A volume of 300 µl of headspace extract or the synthetic blend of *H. uvarum* components was added to 1.2-ml glass vials which served as dispensers. The vials remained without lids and were attached with steel wires inside the traps at the height of the entry holes for the insects (one vial per trap). Preliminary experiments showed that the physical separation of the attractant (dissolved in ethanol and emitted from glass vials fixed at height of the trap openings) in distance to a soapy aqueous drowning solution (in the bottom of the trap) made it difficult to get flies down into the drowning solution. We, therefore, adjusted the set up and applied water 91 ml, tween 0.003 ml, acetic acid 1.6 ml, and ethanol 7.2 ml as drowning solution comparable to the design used by Cha et al. (2013), i.e. vapors from water-soluble acetic acid and ethanol emitted from the drowning solution and merged with the volatiles emitted from the glass vials containing *H. uvarum* headspace extract or the synthetic *H. uvarum* compounds. For reference, using the same drowning solution, we baited traps with methionol as neat compound released from an open glass vial and, in a separate glass vial, acetoin at a concentration of 100 µg/µl ethanol. This reference was formulated based on the bait developed by Cha et al. (2013), however, acetoin was ten times lower concentrated compared to the aqueous acetoin solution used in the original study (Cha et al. 2013), as we had difficulties in dissolving the compound.

Traps baited with the three different treatments (*H. uvarum* headspace extract, synthetic blend of *H. uvarum* volatiles, or the synthetic reference blend) were distributed at two dates (November 14th and 18th, 2019) at three sites of the landscape laboratory. At each site, traps with the three different treatments were placed in a triangular arrangement with ca. 5 m between the traps. Traps were collected after 1 d (November 15th) or 3 d (November 21), respectively, and trap catches were compared based on the number of caught insects per trap and day.

Data Analysis

Analyses were performed using R statistical software (R Core Team 2020). For analyzing the total number of arthropods, drosophilids, and SWD caught in traps baited with *H. uvarum* or Riga, a generalized linear mixed model (GLMM) with a Poisson error distribution (R software package 'lme4') was applied. The specificity of each bait for trapping SWD, either relative to other drosophilids or relative to the total number of trapped arthropod specimen, was analyzed with a GLM fitted with a binomial error distribution for each of the four evaluated trapping periods. For comparison of SWD catches with traps baited with lures that were based on *H. uvarum* headspace extracts, a synthetic blend of *H. uvarum* volatiles, or a synthetic reference blend, a GLMM with a Poisson error distribution was applied followed by a Tukey´s contrast pairwise comparison between the different lures (R software package "multcomp"). The proportion of trapped SWD relative to other drosophilids and to the total of arthropods attracted by the three lures, was analyzed with a GLMM fitted with a binomial error distribution. Sampling dates had no significant effect on the trapping of SWD and data from different dates were therefore combined. The "upwind flight" towards the released volatile stimuli and "contact" with the odor source in the wind tunnel was modeled with a GLM fitted with a binomial error distribution. A Tukey´s contrast test was used for pairwise comparison of fly attraction to different *H. uvarum* headspace dilutions, and for comparison of attraction to different concentrations of the synthetic blend. Residuals were analyzed to verify the distribution of the errors and figures were drawn using "Tidyverse" (R software package "tidyverse").

Results

Hanseniaspora uvarum Attracts SWD More Specifically Than a Wine-Vinegar Based Attractant

Both *H. uvarum* yeast culture and the Riga bait attracted SWD as well as other drosophilids and arthropods (a few arachnids were trapped, in addition to insects) in a four-week experiment at Alnarp, Sweden. Drososan traps with Riga bait captured significantly more SWD (712 *vs* 445; GLMM Poisson, $Z = -8.67$, $P < 0.0001$), other drosophilids (4022 *vs* 596; GLMM Poisson, Z = –43.5, $P < 0.0001$) and arthropods (6790 *vs* 1773; GLMM Poisson, Z = –52.38, $P < 0.0001$) compared to yeast culture. During the trapping experiment, from August to September, we saw an increase in the number of total drosophilids that were trapped with the Riga bait (Table 1). Moreover, the proportion of SWD relative to the number of all other trapped drosophilid flies was significantly higher in traps baited with *H. uvarum* during three of the four trapping periods, while no difference was seen during the first period (Fig. 1, GLM binomial: Date 2019.08.16, $\chi^2 = 2.73$, *d.f.* = 5, $P = 0.098$; Date 2019.08.23, $\chi^2 = 14.98$, *d.f.* = 8, $P < 0.001$; Date 2019.09.02, $\chi^2 = 58.64$, *d.f.* = 8, $P < 0.0001$; Date 2019.09.09, $\chi^2 = 214.43$, *d.f.* = 8, $P < 0.0001$). During the last of the four trapping periods, *H. uvarum* trapped 1.9 times more SWD than all other drosophilid flies together, while on the other hand, traps baited with Riga caught 4.3 times more other drosophilids than SWD.

Headspace Extracts of *H. uvarum* and a Synthetic Blend of Headspace Volatiles Induce SWD Upwind Flight Attraction

Wind tunnel experiments were performed to test the attraction of SWD to *H. uvarum* headspace samples or a synthetic blend of headspace volatiles during a 3 min test period. Samples were dissolved in ethanol and evaporated at the upwind end of the tunnel. Control experiments showed that only few SWD took upwind flight towards ethanol vapor while none of the flies was getting close or in contact with the odor source. When *H. uvarum* headspace was vaporized, most flies took off and flew upwind (Fig. 2). Even a 1:4 and 1:8 dilution of headspace extract induced upwind flight while contact with the odor source was reduced at the highest dilution (Fig. 2, GLM binomial distribution, Multiple Comparison of Means (MCM): Upwind flight, $P > 0.05$; Contact, 1 *vs* 1:4, Z = –0.574, $P = 0.830$, 1 *vs* 1:8, Z = 3.043, $P = 0.006$, 1:4 *vs* 1:8, Z = 2.739, $P = 0.016$). At the highest headspace dose, about 40% (17 out of 43 individuals) of the flies contacted the odor source. When the synthetic blends were tested, again a high number of flies took off for upwind flight. The highest dose of the synthetic blend triggered more upwind flight than a 20-times diluted blend (Fig. 2, GLM binomial distribution, (MCM): Upwind flight, 20 ng/ul *vs* 1 ng/ul, Z = 2.443, $P = 0.039$).

Baits Based on *H. uvarum* Headspace and a Synthetic Blend of Headspace Volatiles Attracted SWD in the Field

Traps baited with *H. uvarum* headspace extract, a synthetic blend of *H. uvarum* volatiles or a synthetic reference blend attracted SWD as well as other drosophilids and arthropods. Overall, the reference blend attracted the highest number of SWD per day (Fig. 3A). Fewer SWD were attracted by the synthetic blend of *H. uvarum* volatiles, and lowest was the average number of SWD in the traps baited with *H. uvarum* headspace extract (Fig. 3A, GLMM Poisson (MCM): *H. uvarum* headspace extract *vs* synthetic blend of *H. uvarum* volatiles, Z = -4.09 $P < 0.001$; *H. uvarum* headspace extract *vs* synthetic reference blend, Z = –7.14 $P < 0.001$; synthetic blend of *H. uvarum* volatiles *vs* synthetic reference blend, Z = 3.365, $P = 0.002$).

However, in comparison to the reference blend or the traps baited with the synthetic blend of *H. uvarum* volatiles, the traps baited with *H. uvarum* headspace extract were significantly more specific in attracting SWD relative to other drosophilids (GLMM binomial (MCM): *H. uvarum* headspace extract *vs* synthetic blend of *H. uvarum* volatiles, Z = 4.36 $P < 0.001$; *H. uvarum* headspace extract *vs* synthetic reference blend, Z = 2.82, $P = 0.013$) or arthropods (Fig. 3B, GLMM binomial (MCM): *H. uvarum* headspace extract *vs* synthetic blend of *H. uvarum* volatiles, Z = 3.88, $P < 0.001$; *H. uvarum* headspace extract *vs* synthetic reference blend, Z = 2.55, $P = 0.028$). In addition to 85 SWD specimen in total, the traps baited with the *H. uvarum* headspace extract attracted only 23 other drosophilids, and 7 nondrosophilid arthropod specimen over the 4 d. In comparison, the traps loaded with the synthetic blend of *H. uvarum* volatiles attracted 122 drosophilid flies in addition to the 148 SWD and 275 arthropods in total, while the synthetic reference blend caught 108 non-SWD drosophilids and 336 arthropods in total, of which 212 were SWD.

Discussion

The yeast *H. uvarum* is a natural attractant of SWD. Trapping experiments in a wood and shrub covered area in Southern Sweden, showed the attraction of SWD to cultures of *H. uvarum* in comparison to the commercially available Riga bait, which is based on a vinegar-wine formulation. In addition, this study illustrates

Table 1. Monitoring for *Drosophila suzukii* (SWD) using Drososan traps baited with *Hanseniaspora uvarum* culture or a vinegar-wine based commercial bait (Riga)

		SWD		Other drosophilids		Arthropods (including all drosophilids)	
Date when trap was collected	Treatment	total	per trap	total	per trap	total	per trap
16.08.2019[a]	Riga bait[c]	20	2.2	505	56.1	889	98.8
	H. uvarum[d]	24	2.0	264	22.0	381	31.8
23.08.2019[a]	Riga bait[e]	36	2,4	525	35.0	1,039	69.3
	H. uvarum[e]	16	1.1	54	3.6	169	11.3
02.09.2019[b]	Riga bait[e]	182	9.1	940	47.0	1,574	78.7
	H. uvarum[e]	93	4.7	114	5.7	505	25.3
09.09.2019[b]	Riga bait[e]	474	23.7	2052	102.6	3,288	164.4
	H. uvarum[e]	312	15.6	164	8.2	718	35.9

Shown are the total numbers of trapped SWD, other drosophilid specimen, and arthropod specimen for four different trapping periods, as well as the average number of daily catches per trap.

[a]traps were collected after 3 d in the field; [b]traps were collected after 4 d; [c]$n = 3$; [d]$n = 4$; [e]$n = 5$

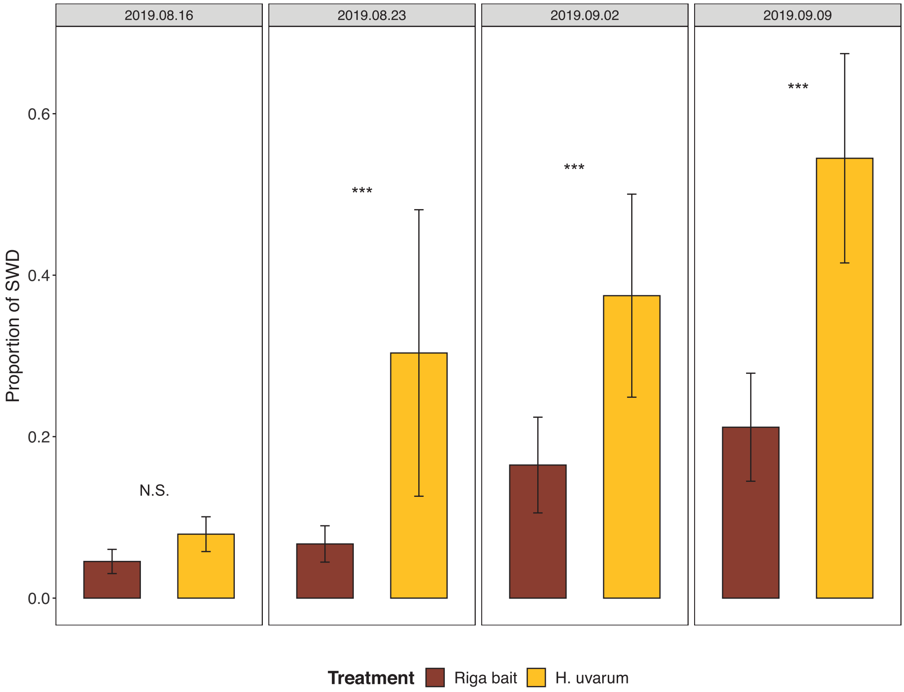

Fig. 1. Proportion (Mean ± SEM) of *Drosophila suzukii* (SWD) flies relative to other drosophilids caught with Drososan traps that were baited either with a yeast culture of *Hanseniaspora uvarum* or a vinegar-wine based commercial attractant (Riga bait). The monitoring was performed in four periods between August and September 2019, in a wood and shrub-covered area at Alnarp, Sweden. The dates give the days when traps were collected from the field after 3 d (for the samples 2019.08.16 and 2019.08.23) or 4 d (samples 2019.09.02 and 2019.09.09) exposure. Asterisks indicate significant difference in the proportion of trapped SWD relative to other drosophilid flies caught between treatments (*** $P < 0.001$). N.S. indicate no significant difference.

an increase of the fly population during a four weeks study in late summer 2019 and confirms the establishment of SWD in Sweden where the invasive pest had been documented previously (Manduric 2017). Comparison of SWD field attraction to *H. uvarum* culture and Riga bait, showed that a higher number of SWD and arthropods in general was attracted by the Riga bait. Similarly, Jones et al. (2021) selected Riga traps as a reference when testing different yeasts including *H. uvarum* strains for SWD attraction in the field. Although a different trap was used, results of the studies are similar in the sense that the Riga bait attracted more SWD than *H. uvarum*. However, our data show, in addition, that *H. uvarum* attracted a higher ratio of SWD relative to other drosophilids and that *H. uvarum* was a more specific lure for SWD than the Riga bait. Interestingly, higher specificity became evident only when the overall number of trapped SWD and other insects were beginning to increase during the second week of our study. Whether selectivity could be improved by increasing the overall attraction to *H. uvarum* at low SWD population densities remains to be studied. The higher number of SWD attracted to the Riga bait might have been caused by a higher effective attraction radius compared to the yeast culture (Byers et al. 1989). While it might be possible to increase the effective attraction radius by increasing the *H. uvarum* dose (Schlyter et al. 1992), attraction of flies from a distance is not necessarily helpful for monitoring SWD in fruit and berry crops.

Other, site-specific characteristics may have biased the captures with these two baits. Lures are known to differ in their selectivity and relative efficacy to attract SWD, depending on site-specific conditions such as the crop (Cha et al. 2018a). Odor backgrounds influence the detectability of attractants positively or negatively, and will thus modulate the insect response towards olfactory stimuli (Schröder and Hilker 2008). Background odors with a different impact on Riga and *H. uvarum* lures may have accordingly influenced the differential attraction of SWD. Wind tunnel tests have demonstrated how background fruit odors can influence the attraction of SWD to *H. uvarum* (Huang and Gut 2021), while on the other hand a green leaf odorant background did not affect SWD upwind flight towards *H. uvarum* (Rehermann et al. 2022). The modulation of SWD attraction to volatile compounds in bioassays and field has previously been discussed as a function of background odors (Cha et al. 2018b).

Encouraged by the greater specificity of *H. uvarum* lures in the field, we sampled *H. uvarum* headspace for a wind tunnel bioassay. While wind tunnel upwind flight attraction to the same *H. uvarum* strain has been shown earlier (Mori et al. 2017), we now demonstrated that it is possible to extract behavioral active compounds from yeast headspace, and that SWD was attracted to the vaporized extract in a wind tunnel. Moreover, dilutions of *H. uvarum* headspace collections illustrated a dose-dependent relation between the headspace release rate and the induced attraction. Although the upwind flight response was not significantly different, the percentage of flies contacting the odor source significantly decreased at the lowest headspace dose. Likewise, we previously showed a dose-dependent

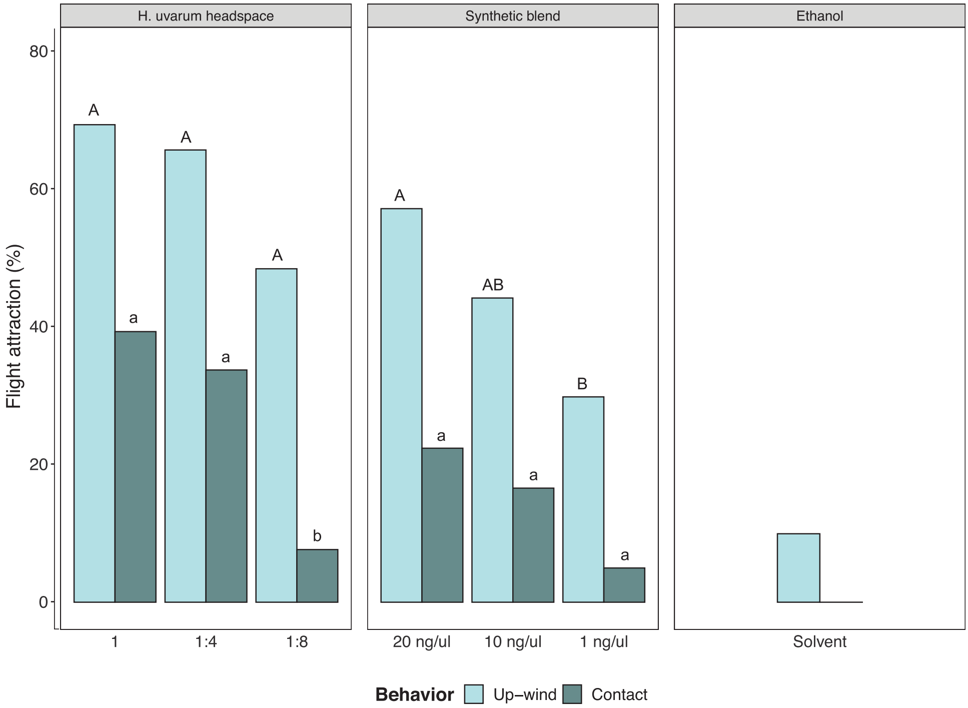

Fig. 2. Upwind flight behavior and contact with the odor source of virgin *Drosophila suzukii* females towards vaporized *Hanseniaspora uvarum* headspace extract, a synthetic blend of seven *H. uvarum* volatiles, and to ethanol. In addition to undiluted *H. uvarum* headspace the extract was tested in dilutions of 1:4 and 1:8. The synthetic blend of *H. uvarum* volatiles was evaluated at three concentrations dissolved in ethanol: 20 ng/µl, 10 ng/µl, and 1 ng/µl. Vaporized *H. uvarum* headspace extract induced strong upwind flight attraction, even at 1:4 and 1:8 dilution. Contact with the odor source was reduced at the highest dilution. Upwind flight to the synthetic headspace blend was highest at 20 ng/µl and decreased significantly at 1 ng/µl. Only few flies exposed to ethanol showed upwind flight, but no contact. Different letters denote significant difference between *H. uvarum* headspace dilutions or the synthetic blend concentrations ($P < 0.05$, uppercase for upwind behavior, lowercase for contact behavior).

decrease of upwind flight attraction to vinegar headspace samples in *D. melanogaster* (Becher et al. 2010). Furthermore, SWD flies were similarly attracted to a synthetic blend of seven components of the *H. uvarum* headspace as to the complete *H. uvarum* headspace extract, and attraction decreased with dilution of the blend. The attraction to the synthetic blend of *H. uvarum* volatiles supported the practicability of the approach to select antennally active compounds for generating a mimic of a behaviorally active headspace sample (Tasin et al. 2006). However, not all antennally active components are essential, and 4 out of 15 compounds were sufficient to reach similar attraction of SWD as an authentic mixture of wine and vinegar (Cha et al. 2014).

Based on our wind tunnel results, we used the headspace and the synthetic blend of *H. uvarum* volatiles in a field test. Preliminary tests indicated that these baits attracted SWD into the traps, but without getting the flies in touch with the drowning solution, which was separated from the vials containing the attractants. We, therefore, followed the procedure by Cha et al. (2013) and added acetic acid and ethanol to the drowning solution. A synergistic effect of acetic acid and ethanol as part of the drowning solution has been shown previously (Landolt et al. 2012a, Cha et al. 2014). With acetic acid and ethanol in the drowning solution and *H. uvarum* headspace extract, or the synthetic blend of *H. uvarum* volatiles as baits in the upper part of the trap, we attracted and captured SWD. However, the contribution of the volatile emissions from the drowning solution to the trapping efficiency of the tested baits still needs to be quantified. It is noteworthy, that acetic acid, which is a common yeast metabolite and is also released by *H. uvarum* (De Benedictis et al. 2011) may contribute to the attraction of SWD to live *H. uvarum*. However, our wind tunnel tests demonstrated that SWD is strongly attracted to a synthetic blend of *H. uvarum* components also without acetic acid.

Ethanol is a suitable solvent for eluting volatiles from headspace filters and moreover does not interfere with SWD behavior in the wind tunnel. We, therefore, used ethanol as a solvent for testing the *H. uvarum* headspace collections or blend of synthetic *H. uvarum* headspace volatiles. We are aware that emission of ethanol from the lures exceeds the natural ethanol emission of *H. uvarum* cultures.

The combination of acetic acid and ethanol is considered to be a basic SWD attractant, and addition of the co-attractants methionol and acetoin enhances trap captures (Landolt et al. 2012a, b; Cha et al. 2018b). The synthetic reference bait in our study attracted the highest number of SWD per trap and day, despite a ten times lower concentration of acetoin compared to the formulation used by Cha et al. (2013). Considering the reported dose-dependent influence of acetoin on SWD attraction, a higher number of SWD might have been captured with the original formulation (Cha et al. 2013 and 2017).

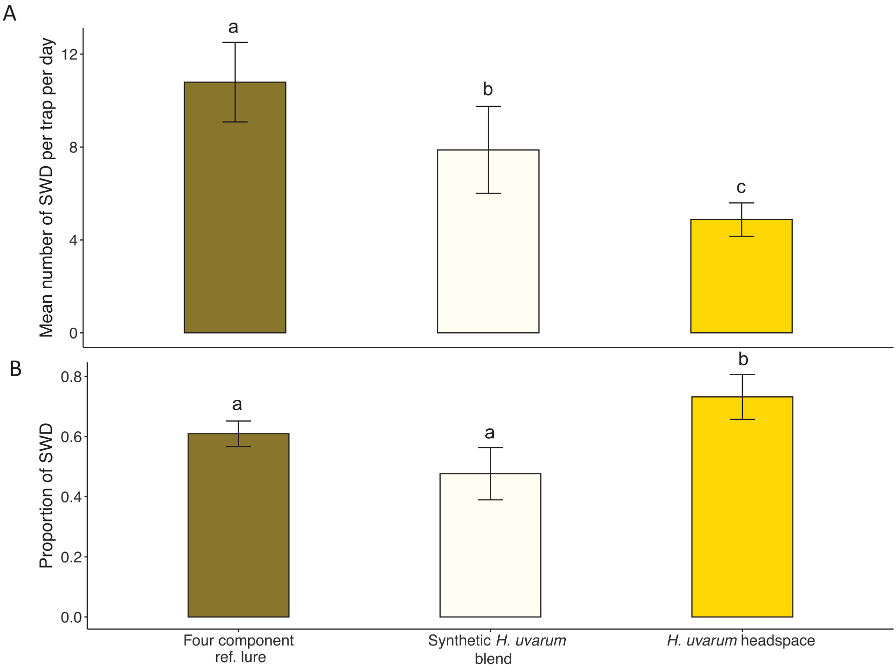

Fig. 3. Field trapping with lures based on *Hanseniaspora uvarum* headspace, a synthetic blend of *H. uvarum* volatiles (Synthetic *H. uvarum* blend), and a four-component reference lure. The assay was performed in November 2019, in a wood and shrub-covered area at Alnarp, Sweden ($n = 6$). (A) Mean number (± SEM) of trapped *Drosophila suzukii* (SWD) per trap per day in Drososan traps that were baited with the three different lures. Traps baited with the reference lure caught significantly more SWD compared to the synthetic blend of *H. uvarum* volatiles or the *H. uvarum* headspace extract. (B) Proportion (Mean ± SEM) of SWD relative to other trapped arthropods for each tested lure. While traps baited with the synthetic blend of *H. uvarum* volatiles caught a similar proportion of SWD as the reference lure, traps baited with *H. uvarum* headspace extract showed a higher specificity for attracting SWD. Different letters indicate significant difference between treatments.

Scheidler et al. (2015) demonstrated a distinct preference of SWD for *H. uvarum* in a laboratory assay, and the two esters isoamyl and isobutyl acetate induced a higher electrophysiological antennal response in SWD, compared to *D. melanogaster*. By adding isoamyl acetate and isobutyl acetate, Cloonan et al. (2019) investigated the possibility to increase fly attraction and selectivity of SWD to a four-component mixture of acetic acid, ethanol, methionol, and acetoin (Cha et al. 2014). However, attraction or selectivity to the four-component mixture was not improved, neither in the laboratory nor in the field, and the investigation of other compound blends and ecologically relevant odors was suggested. In view of the ecological and behavioral relevance of *H. uvarum*, and a strong attraction in the wind tunnel assay, we tested an alternative set of *H. uvarum* volatiles as well as *H. uvarum* headspace, in combination with a drowning solution containing the basic attractants acetic acid and ethanol.

Traps baited with *H. uvarum* headspace extract showed the highest selectivity for SWD in comparison to the synthetic blend of *H. uvarum* volatiles or the reference bait. Our data, in conjunction with an established behavioral response of SWD to *H. uvarum*, support the idea that volatiles from ecologically relevant substrates are a valuable resources for the development of more specific lures. More research is needed to clarify redundancy of active compounds in *H. uvarum* and to optimize and reduce a synthetic mimic to the most essential compounds. Moreover, the relevance of the relative ratios of *H. uvarum* headspace components and their concentrations remains to be investigated. Considering yeast strain specific differences and variability of emitted yeast metabolites, which also depend on growth conditions (Spitaler et al. 2020), our synthetic *H. uvarum* blend is a first attempt, and unlikely a mimic of the behaviorally active *H. uvarum* odors that SWD encounters in nature.

Even the development of population control tactics including attracticides will benefit from the identification of highly specific SWD attractants (Mori et al. 2017, Noble et al. 2019, Bianchi et al. 2020, Spitaler et al. 2020, Rehermann et al. 2022, Spitaler et al. 2022).

First and foremost, there is an immediate need to provide efficient monitoring strategies to growers (Tait et al. 2021). Traps that are easy to use, cost-efficient and reliable in detecting SWD at low population densities prior to fruit infestation are a key pest management tool, and will help to reduce precautionary insecticide applications to protect high value crops.

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All living organisms uses the sense of smell to detect and discriminate odours in their surroundings. In this thesis, I established that humans can discriminate the scent of the cosmopolitan and Zimbabwe strains of *Drosophila melanogaster* and that the invasive species, *D. suzukii* is present in Sub-Saharan Africa. Also, that a female-specific pheromone of *D. melanogaster*, partly induces oviposition avoidance in *D. suzukii* and that a mutualistic yeast, *Hanseniaspora uvarum*, can enhance selectivity of traps.

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