VOLATILE COLLECTION, PERCEPTION, AND BEHAVIOUR – THREE ASPECTS OF INSECT CHEMICAL ECOLOGICAL STUDIES

DOI: 10.54598/006260

Magdolna Olivia Szelényi

Budapest

2025

Ph.D. School

Name: Doctoral School of Horticultural Sciences

Field: Crop Sciences and Horticulture

Head of Ph.D. School: Prof. Dr. Éva Németh Zámboriné
Doctor of the Hungarian Academy of Sciences;
Deputy Director of Institute,
Head of Department of Medicinal and Aromatic Plants
Institute of Horticultural Sciences
Hungarian University of Agriculture and Life Sciences

Supervisors: Dr. Béla Péter Molnár
Senior Research Fellow
Plant Protection Institute
HUN-REN Centre of Agricultural Research;
Lead Scientists
DSM-Firmenich / SwissTPH
† Dr. Gábor Vétek
Associate Professor
Institute of Plant Protection
Hungarian University of Agriculture and Life Sciences

....

Head of Ph.D. School Supervisor

”On the evening of 11 March 2011, RCM first observed the fluorescent orange properties […] at his residence in Sugar Grove, NC.”

(McDonald and Kok, 2014)

1 TABLE OF CONTENTS

2 ABBREVIATIONS

- EAG - electroantennography
- FID - flame ionization detector
- GC - gas chromatography
- GC-EAD - gas chromatography-electroantennographic detection
- GLVs - green leaf volatiles
- GRs - gustatory receptors
- HIPVs - herbivore induced plant volatiles
- IPM - integrated pest management
- IRs - ionotropic receptors
- KIs - Kováts indices
- LOX - lipoxygenases
- MS - mass spectrometry
- MeSa - methyl salicylate
- OBP - olfactory binding protein
- ORNs - olfactory receptor neuron
- ORs - olfactory receptors
- PDMS - polydimethylsiloxane
- PTFE - polytetrafluoroethylene
- SSR - single-sensillum recording
- SPME - solid-phase microextraction
- SRD - sum of ranking differences
- TIC - total ion chromatogram
- VOCs - volatile organic compounds
- DMNT - 4,8-dimethyl-1,3,7-nonatriene
- TMTT - 4,8,12-trimethyl-1,3,7,11-tridecatetraene

3 INTRODUCTION

Chemical ecology plays a crucial role in understanding the complex interactions between organisms and the chemicals in their environment. This is especially relevant in the study of insect behaviour, where volatile compounds emitted by plants or other organisms can significantly influence insects' movement, feeding, reproduction, and communication. The importance of chemical ecology extends beyond basic biological research – it has a direct impact on agriculture and plant protection. In the field of pest management, understanding how insects respond to plant-derived chemicals enables the development of sustainable and targeted control methods, reducing the reliance on broad-spectrum chemical pesticides, which often have harmful environmental effects. Chemical ecology offers promising solutions from identifying key compounds that repel or attract specific insect species to developing environmentally friendly lures and traps that target pests without harming non-target organisms. In this way, chemical ecology is becoming increasingly indispensable to modern agriculture, by helping to protect crops, ensure food security and contributing to the protection of biodiversity.

The urgency of applying chemical ecological principles in plant protection is growing, particularly due to the rise of invasive species. Invasive pests are rapidly spreading due to factors such as climate change and globalization and they cause widespread damage to crops, leading to significant economic losses. With climate change accelerating the spread of these species and intensifying their impact, the need for rapid, effective solutions will likely increase in the future.

However, the complexity of chemical ecology presents significant challenges. The volatile compounds and their roles in mediating interactions vary greatly between species, and understanding theseinteractions – if possible – can take many years. Applied chemical ecology is not well-suited to relying on a single model organism because invasive species frequently emerge, demanding that researchers shift their focus to address new threats. Although the methods for developing protective strategies often remain consistent, the dynamic nature of invasive species requires continual adaptation and exploration of diverse organisms.

To better equip myself for these challenges, I took an unconventional approach during my research. Instead of focusing on a single model species, I joined already existing research projects in various stages. This allowed me to collect relevant data at various points in the process, while also responding to the immediate need to mitigate the impact of emerging invasive species. The dynamic nature of chemical ecology, where newly arrived invasive insects require fast responses, meant that this flexible, multi-faceted approach was essential as the complete workflow can take many years – often longer than the duration of a typical PhD program. Chemical ecologists must

not only be capable of responding swiftly to these threats but also possess the expertise to develop integrated pest management strategies that are both effective and environmentally sustainable.

In this thesis, I present three studies – following the workflow of traditional chemical ecology – that demonstrate a multi-faceted approach to addressing key issues in chemical ecology. Each study focuses on a different stage of the chemical ecology workflow, ranging from the initial step of volatile collection through electrophysiological measurements to validation of the compounds and methods via field trapping.

In the first study, our objective was to identify the optimal combination of adsorbent type and sampling time for volatile compound collection. This investigation utilized three types of adsorbents available within our infrastructure. I joined the project during its initial planning phase, contributing to the experimental design. The second study aimed to determine whether *Metcalfa pruinosa* is capable of perceiving volatile cues. I became involved at the early stage of planning, focusing on the development of volatile collection methods and the design of electrophysiological experiments. In the third study, the objective was to assess whether alive yeast cultures can effectively attract drosophilid species – especially *Drosophila suzukii* – under field conditions. I participated in the initial planning phase, contributing to the design of volatile collection methods and the field trapping protocol.

The research presented here highlights the essential role of chemical ecology in plant protection and agriculture and underscores the importance of adaptive and flexible approaches in combating the growing problem of invasive species.

4 OBJECTIVES

This thesis consists of three parts, each corresponding to one of three previously published studies. The primary objectives of these studies are outlined below:

1. **Comparison of various techniques for volatile collection from headspace**: As the set of compounds collected is heavily depends on the techniques used, the first objective was to identify the volatile collection setup collecting the most amound of volatiles.
2. **Development of an electrophysiological measurement method for a hemipteran species**: Behavioral studies indicate that olfaction plays a crucial role in the colonization of new territories and the location of suitable habitats. However, electrophysiological approaches to studying the olfactory systems of vector species within the Auchenorrhyncha group remain relatively understudied. The second goal was to establish a reliable method for conducting electrophysiological measurements on the hemipteran *M. pruinosa*, with the aim of identifying the volatile compounds that are perceived by the insect.
3. **Development of novel, yeast-based, field-effective attractants for *D. suzukii***: The association between *D. suzukii* and yeasts, particularly *Hanseniaspora uvarum*, presents a promising opportunity for lure development. However, limited data exists regarding the volatilome and attractiveness of live yeasts and yeast-infested fruit substrates under realistic field conditions. High specificity is important, as otherwise non-target species could be impacted, which could, in turn, adversely affect biodiversity. To address this, various yeast species and their emitted volatiles were tested to identify the most effective and most selective attractant.

Each of these studies aims to contribute to the advancement of pest management strategies by addressing specific chemical ecological challenges related to invasive insect species and their interactions with plants.

5 LITERATURE REVIEW

5.1 From Phenomenon to Product – Harnessing Chemical Ecology for Innovative Trapping Solutions

This journey – often spanning several years – involves observing phenomena in nature, as highlighted by the motto of this thesis, and posing the fundamental question: "Why?"

Chemical ecology provides valuable insights into the origins, functions, and significance of natural chemicals that mediate interactions between organisms. By studying these interactions, chemical ecologists aim to leverage them for practical applications, particularly in plant protection, where such natural chemical cues can be used to combat pests more effectively.

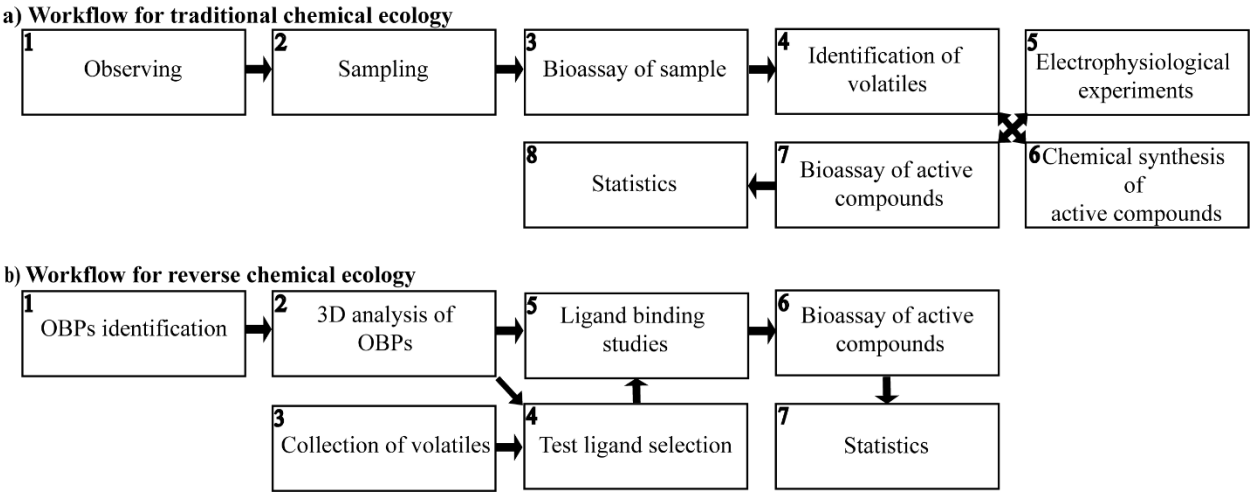

Figure 1 *Workflows of traditional and reverse chemical ecology approaches (based on Barbosa-Cornelio et al. (2019)).*

Chemical ecologists begin their research in either of two ways (Figure 1). The traditional chemical ecology approach and the reverse chemical ecology approach. The traditional chemical ecology approach workflow consitst of the following steps: (1) the first step is to observe interactions between organisms that suggest the involvement of chemical signals (semiochemicals); (2) samples of volatile compounds or extracts are collected from the environment or organisms involved in the interactions; (3) the collected extracts or volatiles are tested on insects through bioassays to assess their behavioral or physiological effects; (4) the active compounds from these samples are identified using various analytical techniques; (5) electrophysiological techniques are used to determine which of the identified volatiles are detected by the insect's sensory organs; (6) the selected active compounds are then tested, either individually or in mixtures, in behavioral assays to study their effect on insect behaviour; (7) the active compounds could be synthesized in larger quantities, allowing further testing and potential applications in pest control; (8) finally, data from the bioassays, identification, and behavioral

studies are statistically analysed to confirm the efficacy and role of the active compounds (Barbosa-Cornelio et al., 2019).

The reverse chemical ecology approach workflow consists of the following steps: (1) the reverse approach starts by identifying and expressing olfactory binding protein (OBP) genes from the target insect using molecular or bioinformatics tools, as OBPs are crucial for detecting volatiles; (2) 3D structures' of OBPs are analysed to understand how they interact with semiochemicals; (3) at the same time test semiochemicals (either isolated or synthetic volatiles) collected from commercial or in-house libraries for further testing; (4) select ligands – often through *in silico* methods – for further experimentation; (5) selected ligands are tested in vitro to study their binding affinity to the OBPs, determining which ligands have the strongest interactions; (6) the ligands that show high binding affinity are then tested on the insects *in vivo* to verify their effects on insect behaviour; (7) statistical analysis is performed to assess the significance and reliability of the results obtained from the binding studies and behavioral experiments (Barbosa-Cornelio et al., 2019).

Both approaches ultimately aim to uncover chemical cues involved in insect behavior, but the conventional approach begins by identifying active chemicals in the environment, while the reverse approach starts with molecular-level tools to find which compounds may interact with the insect's chemosensory system.

Chemosensation is the sensory process through which insects detect and respond to chemical cues in their complex environment. This includes the detection of volatiles which are essential for locating suitable habitats, finding food, identifying mates and avoiding predators or harmful substances. Insects possess specialized chemosensory organs, such as antennae, modified mouthparts which feature sensory hairs containing receptors. These receptors include olfactory receptors (ORs), ionotropic receptors (IRs) and gustatory receptors (GRs) all of which detect and respond to specific chemical compounds in their environment. Chemosensation is crucial for the survival, reproduction, and ecological interactions of insects, making it a key area of study in fields such as entomology and chemical ecology (Hansson and Wicher, 2016).

Chemical signals, or chemostimuli, are molecules released from different sources like soil, plants, or animals. These molecules may be volatile or dissolved in solution. Volatile molecules are usually sensed through ORs, while dissolved molecules are detected by GRs (Hansson and Wicher, 2016).

5.1.1 Volatile Collection and Chemical Analysis

Different sampling methods can significantly affect the characterization of volatile compounds and alter the results (Agelopoulos and Pickett, 1998; Castellan et al., 2024). Several

volatile extraction methods are available, but in recent years, stir-bar sorptive extraction (SBSE), headspace sorptive extraction, solid-phase microextraction (SPME), and dynamic headspace system sampling techniques have gained significant popularity. These methods enable the collection of volatiles either within or above the target matrix using commonly employed adsorptive materials (Nogueira, 2015).

Volatile sampling methods can be classified into two groups based on the carrier phase used to transfer the volatiles: dynamic and static sampling. These methods are simple and versatile for use in both laboratory and field environments, whether above or below ground. Polydimethylsiloxane (PDMS) tubing, in particular, can be used in large quantities and stored at freezing temperatures without the need for organic solvents. These methods allow for controlled collection and pre-concentration of volatile organic compounds (VOCs), enabling both qualitative and quantitative analysis with repeatable sample results. However, quantitative analysis is restricted, and adsorbents may preferentially capture certain analytes (Tholl et al., 2021).

Dynamic sampling methods, including pull/push-pull systems and adsorbent traps – both thermal and solvent desorption –, offer controlled collection and pre-concentration of VOCs, with the potential for qualitative and quantitative analysis in diverse environments. The use of miniature sampling devices is an advantage, though these methods often require additional equipment, such as pumps, flow meters, and plant organ encasement, which complicates field use. Organic solvents are commonly employed in solvent desorption; however, their use comes with several challenges (Tholl et al., 2021). They can be costly, potentially toxic if not handled properly, and may contain contaminants that could inadvertently affect the result of the electrophysiological experiments (Tasin et al., 2012). Adsorbent traps can show a preference for certain analytes. Dynamic headspace system offers a broad selection of ready-to-use adsorbents such as activated charcoal, Porapak Q, Carbotrap, HayeSep, and Tenax. The selectivity for specific compounds depends on the type of adsorbent used, making it essential to align the adsorbent with the research question (Ochiai et al., 2014; Diez-Simon et al., 2020).

The static sampling technique relies on the adsorption and thermal desorption of volatile compounds using an inert fiber coated with adsorbents of varying polarity and thickness, depending on the type and concentration of compounds being collected. Typically, solid-phase microextraction (SPME) fibers are inserted into a hot injector port of a gas chromatograph (GC) to release the trapped volatiles. Recently, a more advanced SPME device called the SPME Arrow (CTC Analytics AG, Zwingen, Switzerland) was developed, providing enhanced stability by fully enclosing the fiber in a needle, thus protecting it from contamination and physical damage (Feijó Barreira et al., 2018). Stir-bar sorptive extraction (SBSE, Twisters) is another alternative to SPME that offers even greater adsorbent capacity. Although SBSE is limited to non-polar compounds

due to PDMS's characteristics, it has proven effective for analyzing VOCs in various environments. While these static techniques are ideal for qualitative VOC analysis, quantitative accuracy can be challenging (Tholl et al., 2021).

In the highly complex VOCs, volatile compounds are typically separated by gas chromatography (GC), and the separated volatiles are analysed either by mass spectrometry (GC-MS) and/or a separate flame ionization detector (GC-FID).

To ensure accurate VOC analysis, optimizing the sampling process is crucial.

5.1.2 Insect Olfaction and Electrophysiology

Around the beginning of th 20th century, behavioural experiments indicated that the antenna is the main olfactory organ in insects.

Antennae are crucial multimodal sensory organs, containing sensilla not only for olfaction but also for taste, mechanoreception, hygroreception, thermoreception, and sometimes CO_2 detection. Insect antennae come in many different shapes and in many different sizes. In higher insects, they are subdivided into three parts. The basal segment, or scapus, is connected to the head capsule through an elastic membrane and a ball-and-socket joint, enabling movement facilitated by four muscles, two of which extend into the second segment. The second segment, the pedicel, is linked to the scapus via another elastic membrane and is moved by two muscles that pass through the membrane separating it from the scapus. The long flagellum forms the main part of the antenna and carries most of the sensilla. It is subdivided into flagellomeres, or annuli, which are not true segments. The flagellum has no muscles and is passively moved by the pedicellus (Schneider, 1964).

The smallest functional sensory unit in insect olfaction is the sensillum, which is a complex structure composed of bipolar olfactory receptor neurons (ORNs), auxiliary cells, and cuticular elements. An olfactory sensillum consists of one to several bipolar ORNs surrounded by a special set of concentrically arranged auxiliary cells that form cuticular, subcuticular, and glial elements. While the number of neurons varies depending on the type of sensillum and the species, the number of auxiliary cells remains relatively constant.

There are several reports on olfactory sensilla on the mouthparts (e.g. sensilla basiconica: (Liu et al., 2021)).

The olfactory sensory neurons' (OSNs) dendritic structures that express chemosensory receptors are responsible for binding odor molecules. In insects, volatile chemical detection primarily occurs through two distinct receptor types: odorant receptors and ionotropic receptors (Hansson and Stensmyr, 2011; Sharma et al., 2015). Olfactory transduction begins with the binding of a ligand to an OR (Joseph and Carlson, 2015). The number of ORs varies significantly

among insect species, with 62 identified in Drosophila, 79 in Anopheles, 131 in Aedes mosquitos, 157 in Apis, 48 in Bombyx, and 265 in Tribolium. The receptor potential generated by this binding is converted into action potentials, which are transmitted to the central nervous system via the axon. The axons of individual ORNs terminate in an olfactory glomerulus (Sato and Touhara, 2009). The glomerulus contains the branches of local interneurons and the dendrites of projection neurons, which relay the processed information to higher brain regions (Hansson and Stensmyr, 2011). However, the extent to which volatile compounds elicit behavioral responses is influenced also by the physiological state of the insect (McCormick et al., 2016)

Definitive proof that indeed the antenna is the main olfactory organ came with the electroantennography. Electroantennography (EAG) was first described in 1956 by Schneider and Hecker during their efforts to identify a volatile compound from the silkworm, *Bombyx mori* L.. Schneider observed slight variations in voltage between the tip and the base of the insect antenna when stimulated with pheromones. He noted that the amplitude of the antenna response signal increased with both the concentration of the volatile compound and the air flow rate (Schneider and Hecker, 1956; Schneider, 1957). Over the past 60 years, two main types of antennal recordings have been utilized for studying insect olfaction: EAG and single-sensillum recording (SSR). EAG involves recording the total field potentials across the antenna elicited by all of the activated receptor neurons. In contrast, SSR introduced by Einzelnen in 1962 and later by Schneider & Boeckh in 1962, involves recording the spiking activity elicited by the olfactory receptor neuron(s) of a single sensillum. SSR offers several advantages over EAG, as the signal is easier to interpret due to the clear timing of the action potentials traveling through the antennal nerve, which deliver the detected sensory information to antennal lobe neurons in the brain. This method reliably measures the ORN's output and correlates directly with some labeled-line behaviors and the activity level of specific ORN classes. However, the arbitrary sampling of individual sensilla in SSR provides incomplete access to the functions of the olfactory organ. First, exploring the antennal responsiveness requires pooling data from many individuals, which prevents the estimation of interindividual variability. Secondly, some classes of ORNs could be completely disregarded, leading to an incomplete understanding of the olfactory system (Jacob, 2018), as it was suggested by Arn et al., (1983) in the case of *Agrotis segetum*.

EAG setup involves fixing the antenna (or in some cases with the head still attached) between the recording and reference electrodes. These electrodes are either filled with ringer solution – an electrolyte solution similar in ionic composition to hemolyph –, or they are solid electrodes made from tungsten or other metals such as silver. Upon receiving a chemical stimulus, the electrical potential fluctuates across the antenna, which can be detected. The EAG signal results from the summation of the activity of many ORNs. When exposed to a stimulus containing more

then one volatile, the EAG response integrates signals from different classes of chemosensory receptors, reflecting the collective neural activity. When EAG is coupled to GC, it becomes possible to assess complex mixtures of volatiles. In gas chromatography-electroantennographic detection (GC-EAD), the column flow is divided using an Y-shaped splitter. A portion of the injected sample is directed to a FID or a MS or, in case of 3-ways cross, even to FID and MS at the same time (Shuttleworth and Johnson, 2022), while another part is directed to the antenna for electroantennographic detection. This setup allows for simultaneous chemical separation and detection of compounds that specifically activate insect antennae (Kennedy and Moorhouse, 1969; Myrick and Baker, 2012).

The amplitude of the EAG response depends both on the receptor potential response of individual ORNs and on the density of responsive ORNs in the vicinity of the recording electrode (Kaissling, 1986; Bigiani et al., 1989). Typically, the apical end of the antenna, which is sensitive to odor molecules, is placed on the recording electrode, while the base is placed on the reference electrode. However, Jacob (2018) showed that the position of the recording electrode can have a significant effect on the results, highlighting the potential for further development of the EAG methodology. The exact method of antennal preparation should depend on the insect studied, especially on the shape of the antennae and localization of sesillae.

For all its advantages and disadvantages EAG and GC-EAD are highly useful for detecting molecules that have physiological effects on insects, aiding in the identification of compounds which have behavioural effect and thus potential as semiochemicals.

5.1.2.1 Antennal Morphology in Hemimetabolous Insect

In hemimetabolous species, antennal structural differences are less distinct than in holometabolous species. During nymphal development, flagellum elongation occurs through the addition of a few flagellomeres (Guglielmino and Virla, 1997; Keil, 1999). In fulgoroid hemipterans, olfactory sensilla are mainly located on the scape and pedicel, increasing during morphogenesis (Romani et al., 2009; Wang et al., 2018). Studies show that the peripheral detection abilities of the spotted lanternfly (*Lycorma delicatula*) differ between the fourth nymphal stage and adulthood (Moon et al., 2011). Additionally, Auchenorrhyncha species show sex-specific distributions of olfactory sensilla (Wang et al., 2018; Zhu et al., 2019).

The citrus flatid planthopper (Metcalfa pruinosa) is polyphagous, using over 300 host plants (Alma et al., 2005; Seo et al., 2019). Nymphs disperse after hatching but settle after the fourth stage, with a second migration occurring when adults emerge. While nymphs and adults show no significant differences in host preference, olfactory plasticity may explain seasonal shifts in host usage.

M. pruinosa is known to carry several phytoplasmas and a species of *Pseudomonas* (Guadagnin et al., 2000; Landi et al., 2007; Donati et al., 2017; Kirkpatrick et al., 2018), effectively vectoring some of these plant pathogens that cause symptoms leading to plant decline and death (Weintraub and Beanland, 2006; Donati et al., 2017; Mergenthaler et al., 2020). Despite its ecological and agricultural significance, factors influencing host plant selection in M. pruinosa remain poorly understood. Previous studies suggest that it may detect plant volatiles (Youn, 2002; Moon et al., 2011; Riolo et al., 2012; Zhang and Chen, 2015; Coll Aráoz et al., 2019; Anastasaki et al., 2021).

5.1.3 Behavioural Analysis and Field Work

What is a behaviour? It refers to movement of a part of an organism, the entire organism itself, or a group of organisms, as well as the physiological basis of such movement (Bell and Cardé, 2013). How can one measure behaviour?

In laboratory assays, the choice of technique should depend on the insect's behavior. For insects that crawl, walk, or jump, olfactometers (also known as "olfactory response meters") are commonly used. Olfactometers have been in use for over a century and play a fundamental role in experimental chemical ecology, enabling researchers to study insect responses to various chemical stimuli. These devices allow researchers to study the behavioural responses of insects in a controlled environment to various olfactory stimuli facilitating research in chemical ecology and the identification of behaviour-modifying compounds as attractants or repellents. Olfactometers come in several versions, but the most commonly used are the two-way (Y-tube), four-way (four-arm), and six-way (six-arm) olfactometers. In still air olfactometers typically consist of an enclosed arena in which one or more chemical stimuli are introduced before adding the study subject. In the others a continuous airflow carries scent molecules from the source. Chemical stimuli are typically introduced into the arena as volatiles originating from either biological material or purified synthetic chemicals. To minimize directional bias caused by external stimuli such as light or temperature, it is essential to periodically rotate the entire olfactometer during the experiment. Additionally, increasing the number of treatment arms in four- or six-arm olfactometers to two, with these treatment arms being identical but directly opposite each other, can help mitigate such biases (Haynes and Millar, 1998; Roberts et al., 2023).

Wind tunnel behavioural studies are valuable for examining the responses of flying insects to odorants. Wind tunnels allow for the observation of anemotaxis, where insects navigate their flight toward odorants within a controlled laboratory environment. Insect wind tunnels come in various sizes and shapes and can operate with airflow based on either suction or blowing principles, providing flexibility in experimental design. Natural odor sources, such as plant parts,

as well as synthetic odors from dispensers, can be directly introduced into the flight arena for testing and observation (Baker and Cardé, 1984; Knudsen et al., 2018).

The last stage of developing working lure is to take volatiles out to the field. In the field, insects encounter numerous additional olfactory, visual, and acoustic stimuli that can influence their behaviour. The exact laboratory results are often not repeatable in the field because of these uncontrolled factors. The success of field experiments is influenced by variables such as the actual population size of the target species in the experimental area or weather conditions, which are beyond experimental control. To increase the reliability of field study results, experiments should be conducted at multiple locations and, if possible, over several flight periods, although this requires additional work and time. In field experiments, common questions are determining which odorant or combination of odorants, as well as the release rates – amount emitted per unit time – or ratios, are needed to elicit a behavioral response. The formulation and choice of dispenser significantly influence these quantitative aspects, affecting the ratios of emitted odorants, their release rates, and the total duration of the release. Selecting the appropriate trap design is crucial for capturing the highest proportion of the target species approaching the bait, by leveraging the species' behavioral patterns (Cardé and Elkinton, 1984; Vuts et al., 2018).

Odor-based methods present a promising alternative to traditional pest management approaches, such as chemical insecticides or mechanical traps by selectively attracting or disorienting target insects. Often there is a tradeoff between attractiveness and specificity, where the most attractive lure in terms of total catch may not necessarily be the most attractive in terms of specificity and, consequently, sustainability. An overly broad attractiveness might result in the capture of both target and non-target species, compromising ecological balance, while a lure with greater specificity could enhance sustainability by minimizing its impact on non-target organisms and reducing unintended consequences within the ecosystem. Unfortunately, bycatches are often underreported, making it difficult to accurately assess the sustainability of these methods (Larsson Herrera et al., 2020).

5.2 Plant Protection Based on Chemical Ecology

As climate change brings higher temperatures, effectively managing both native and invasive insect populations become increasingly critical. Relying solely on increased use of insecticides is not a sustainable solution (Md Meftaul et al., 2020). Insects have extremely advanced olfactory systems and rely on their sense of smell to perform many crucial behaviors, such as breeding, oviposition, prey location, and defense. This characteristic of insects implies that semiochemicals could be used in plant protection. Semiochemical-based strategies have gained prominence as a crucial element of integrated pest management (IPM). The strategic application

of pheromones and other semiochemicals offers a promising path toward sustainable, area-wide pest management. This approach not only meets the immediate need for effective pest control but also plays a vital role in enhancing food security for the growing global population (Witzgall et al., 2010; El-Ghany, 2019).

Pheromones – chemical signals secreted or excreted by an organism that triggers a specific behavioural or physiological response in individuals of the same species – are particularly effective at low population densities, as they specifically target the pest species without negatively impacting natural enemies. This selective action promotes a long-term reduction in insect populations, something that is often difficult to achieve with conventional insecticides (Witzgall et al., 2010). Pheromones are utilized for insect pest control through two main approaches: indirect and direct control. Indirect control involves monitoring and optimizing spray timing (Burkholder and Ma, 1985). Direct control encompasses mass trapping (Beroza and Knipling, 1972), mating disruption (Ioriatti et al., 2004) and lure-and-kill methods (El-Sayed et al., 2009; Klick et al., 2019). Furthermore, pheromones used in live-catching traps play a crucial role in assessing the genetic diversity of pest populations (Carter et al., 2009) for conservation of rare and threatened insects (Larsson, 2016). Though there are many cases when using pheromones are not suitable; e.g. the pheromone (blend) is still unknown; there is no pheromone at all; or species specifiy in undesired in case of entomological surveys (Szanyi et al., 2024).

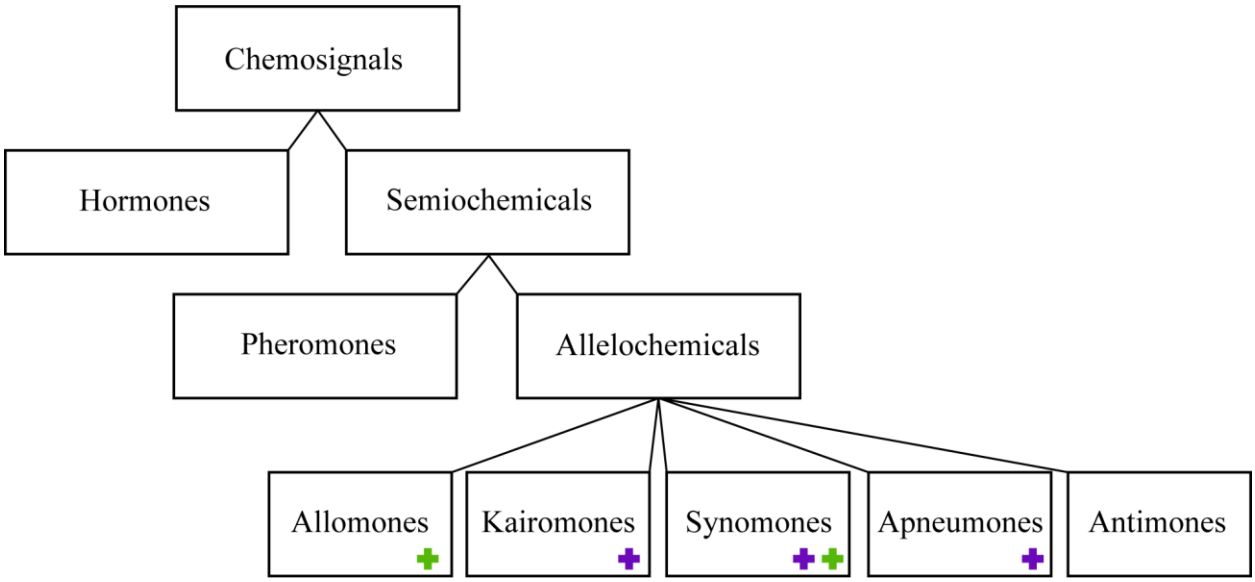

Figure 2 *Hierarchical classification of chemosignals based on the relationship between the emitter and receiver, denoted by symbols indicating benefits: green + signifies benefit to the emitter, while purple + indicates benefit to the receiver (based on Hansson & Wicher (2016)).*

Allelochemicals (Figure 2) are substances that facilitate interspecific communication, transmitting chemical messages between different species. Essentially, these substances are primarily emitted by individuals of one species and percieved by individuals of another species. They are categorized

into five distinct groups: allomones, kairomones, synomones, antimones, and apneumones.(El-Ghany, 2019).

- Allomones: Substances released by one organism that prompt a response in another species, typically beneficial to the emitter, like toxic compounds deterring herbivores (Mayer et al., 2008).
- Kairomones: Emitted by one organism to stimulate a beneficial response in another species, such as attracting predators or hosts, sometimes overlapping with allomones or pheromones (Metcalf, 1985; Murali-Baskaran et al., 2018).
- Synomones: Mutualistically beneficial to both the emitter and receiver, like floral scents attracting pollinators or plant volatiles recruiting natural enemies of pests (Turlings et al., 1990).
- Antimones: Detrimental to both the emitter and receiver, triggering a repellent response in encountering individuals of different species.
- Apneumones: Emitting from non-living sources, prompting a favorable response in one species but potentially harmful to others. Rare instances of apneumones, such as hexanal and 2-methyl-2-butanol released from rabbit stools, have been documented to attract sandfly females for oviposition (Dougherty et al., 1995).

5.2.1 Allelochemicals Used in Plant Protection

5.2.1.1 „Cry for help"

To compensate for their immobility, plants have evolved various mechanisms for interacting with their environment, including the release of an array of volatile compounds from their leaves, flowers, and fruits into the atmosphere and from their roots into the soil (Dudareva et al., 2006). Plants exhibit one of the highest diversities of VOCs, only in floral scent 1700 compounds are reported (Knudsen and Gershenzon, 2020). These metabolites encompass various classes, including terpenes, phenolics, benzenoids, nitrogen- and sulfur-containing compounds, and fatty acid derivatives (Tholl et al., 2021). In nature, volatile organic compounds (VOCs) play crucial roles in plant adaptation and survival. They are often emitted directly from flowers to attract pollinators, facilitating reproduction and ensuring species continuity. In response to herbivore attacks, plants activate their defence systems by releasing a range of volatiles. These compounds are typically stored in specialized cells and structures such as glands and ducts (Dudareva et al., 2006; Turlings and Erb, 2018). Upon mechanical injuries, ie. resulting from insect feeding, C6 aldehydes, alcohols and their esters are immediatly released, but there are other, de novo synthetised volatiles – like 4,8-dimethyl-1,3,7-nonatriene (DMNT), 4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), (*E*)-*β*-ocimene – which may be released later after the attack (Pare and

Tumlinson, 1997; Turlings and Erb, 2018). The composition of these herbivore induces plant volatiles (HIPVs) depends on a variety of factors even within a species (Dicke and Baldwin, 2010). The role of these volatiles is binary: some volatiles notify neighboring plants (Heil and Karban, 2010), while others, or the same compounds, alert the natural enemies of the attacking herbivore (Turlings and Erb, 2018).

Green leaf volatiles (GLVs) are synthesized from oxygenated fatty acids present in plastid membranes via an enzymatic cascade involving lipoxygenases (LOX) and hydroperoxide lyase. This process leads to the production of (*Z*)-3-hexenal, which is subsequently converted into (*Z*)-3-hexenol by alcohol dehydrogenase and further transformed into (*Z*)-3-hexenyl acetate by alcohol acyltransferase. GLVs function as rapid and relatively specific signals of herbivore attack. Transgenic plants deficient in GLVs due to LOX2 silencing exhibited increased susceptibility to herbivory in field conditions and produced fewer flowers in the presence, but not the absence, of these herbivores (Schuman et al., 2012).

Methyl salicylate (MeSa) is a volatile plant defense hormone typically induced in response to biotrophic pathogens and certain sap-sucking insects. Commercially available dispensers containing MeSa and other attractants for beneficial insects are already in use (Lee, 2010).

Cis-jasmone is a volatile plant hormone that has been specifically investigated for its potential in field applications. Cis-jasmone triggered a chemical defense response in soybeans, which was comparable to the response observed from stink bug damage. The primary compounds induced by cis-jasmone included camphene, myrcene, (*E*)-*β*-ocimene, MeSa, and TMTT. It has been successfully used to attract egg parasitoids of stink bugs, *Telenomus podisi* in soybean crops (Moraes et al., 2009).

Field evidence supporting the potential of genetically modified plants comes from the successful genetic manipulation of maize to restore the release of (*E*)-*β*-caryophyllene, a root-derived attractant for entomopathogenic nematodes leading to enhanced protection against rootworm (Rasmann et al., 2005; Degenhardt et al., 2009). However, maize plants releasing (*E*)-*β*-caryophyllene also become more attractive to pests (Robert et al., 2013). Behavioural assays indicate that the release of *β*-myrcene and *β*-caryophyllene volatiles from dispensers enhances the effectiveness of *Encarsia formosa* as a biological control agent against *Bemisia tabaci* whiteflies in glasshouse production systems (Chen et al., 2021).

β-ocimene, one of the most extensively studied and significant HIPV, has been shown to effectively attract various predators, including parasitic wasps such as *Aphytis melinus*, lady beetles, and green lacewing larvae, *Chrysoperla carnea*. This attraction has facilitated the biological control of key pests, including the california red scale, *Aonidiella aurantii* and the aphid, *Myzus persicae* (Laznik and Trdan, 2018; Mohammed et al., 2019).

However, the success using volatiles as lures requires careful consideration of the synchronicity between crop pests and their natural enemies. The "attract and reward" pest control strategy combines the attractant effects of synthetic HIPVs with companion plants that offer a "reward" to extend the survival of parasitoids and predators in the absence of hosts or prey (Legaspi et al., 2020; Mohammed et al., 2020).

5.2.1.2 Host organism volatiles

Insect responses to plant volatiles vary, being either attractive for adapted herbivores or repellent for non-adapted ones. However, classifying plant volatiles as attractants or repellents isn't standardized due to fluctuations in insect behaviour responses, which depend on the concentration of these volatiles. The same chemical compound can function as an allomone, shielding the plant from certain herbivores, while acting as a kairomone that stimulates the feeding behaviours of other herbivores, depending on the intricacies of mutations and coevolutionary processes (Metcalf, 1985).

Regarding hemipteran species, MeSa, 1-octen-3-ol, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate, (*E*,*E*)-*α*-farnesene, methyl benzoate, DMNT, and (*Z*)-3-hexenyl butyrate was found to attract *Lycorma delicatula* (Derstine et al., 2020). Volatile unsaturated diterpene hydrocarbon alone or in combination with one or more of the minor compounds emitted by seedlings of Brassica species serve as host location cues for *Bagrada hilaris* (Guarino et al., 2018). Evidence indicates that aphids utilize both host-specific volatiles and host-specific ratios of different compounds, blends of ubiquitous volatiles for locating their hosts. Isothiocyanates are characteristic volatile compounds almost exclusively in the order Brassicales. Specialist aphids feeding on species of Brassicales are attracted to different type of isothiocyanates (Dilawari and Atwal, 1989; Nottingham and Hardie, 1993), but at the same time isothiocyanates had a repellent effect on *Aphis fabae*, an aphid struggling to survive on brassicaceous plants (Nottingham et al., 1991; Isaacs et al., 1993). *Phorodon humuli* were attracted to (*E*)-2-hexenal and *β*-caryophyllene when offered at the same ratio as that identified from hop plants, though both compounds are fairly widespread in the plant kingdom and not specific to hop (Campbell et al., 1993).

5.2.1.3 Microbial volatiles against invasive species

Allelochemicals can be used in push-pull strategy. The spotted-wing drosophila (*Drosophila suzukii* (Matsumura)) is a highly polyphagous vinegar fly, endemic to East Asia (Asplen et al., 2015). Female *D. suzukii* possess serrated ovipositors capable of puncturing the fruit epidermis, enabling oviposition into ripening soft-skinned and stone fruits. Both larval feeding and secondary microbial infestation result in significant fruit damage, consequently leading to substantial economic losses (Knapp et al., 2021). Since the initial stages of the intercontinental

spread of *D. suzukii*, fermentation baits have been employed for monitoring purposes (Bueno et al., 2020). The yeast *H. uvarum*, which is closely associated with *D. suzukii*, has a strong attractant effect on these flies, encouraging them to feed on food enriched with yeast. Formulations containing spinosad applied in the greenhouse demonstrated that both *H. uvarum* culture and the yeast cell-free supernatant from a centrifuged culture increased fly mortality and reduced the number of eggs laid compared to the unsprayed control (Spitaler et al., 2020; Rehermann et al., 2022). One odor, 1-octen-3-ol was used as an aversive chemical stimuli for *D. suzukii* successfully in laboratory and in field to reduce the oviposition (Wallingford et al., 2016; Wallingford et al., 2018).

6 MATERIALS AND METHODS

6.1 Comparison of Multiple Techniques for Sampling Optimization

6.1.1 Plant material

In these set of experiments, *Lactuca sativa* 'Rivalda' was used, supplied by Rijk Zwaan Budapest Ltd. (Budapest, Hungary). This variety was selected due to its excellent shelf-life and remarkable disease resistance towards soil born pathogens. Seeds were sown in 1.7 L pots using potting soil (Kekkilä DSM 3W, Kekkilä-BVB Vantaa, Finland) in in a greenhouse under natural light, with temperature conditions maintained between 18-25°C and relative humidity approximately 40%. To prevent soil volatiles from contaminating the headspace samples, the soil was covered with thin layers of aluminum foil post-germination, allowing only the lettuce plant to protrude through a 2-cm diameter hole at the center.

6.1.2 Volatile Collection

Volatile were collected from the parts above gound level of lettuce plants on the 60th day post sowing. Each plant was encased in a Nalophane NA foil tube (20 μm; Kalle Hungaria Kft., Budapest, Hungary) one day prior to measurements. A continuous flow of charcoal-filtered air (1 L min^{-1}) was drawn through the system using a vacuum pump (Thomas G 2/02 EB, Gardner Denver Thomas GmbH, Fürstenfeldbruck, Germany). Each of the volatile traps used contained 50 mg of Porapak Q (80-100 mesh), HayeSep Q (60-80 mesh), and Carbotrap (20-40 mesh) adsorbents (Supelco, Sigma-Aldrich, 595 North Harrison Road, Bellefonte, PA, USA). Headspace sampling of volatiles took over the durations of 1, 2, 4, or 6 hours in a closed loop system. Temperature was consistently maintained at 25 ± 1 °C. Prior to each collection, the adsorbent filters were cleaned as outlined by Molnár et al. (2015). The adsorbed volatiles were subsequently extracted with 300 μL of n-hexane (>99%, Sigma-Aldrich) into 1.5 mL vials and stored at -18 °C until analysis via GC-MS.

6.1.3 Analytical Measurements

An Agilent 6890 gas chromatograph coupled with a 5975 C MSD mass spectrometer was employed to analyze the collected volatiles. A non-polar HP-5 UI ((5%-phenyl)-methylpolysiloxane; 30 m × 0.25 mm × 0.25 μm film; J&W, Santa Clara, CA, USA) capillary column was used for the analysis. 1 μL of each sample was injected into the GC injector, which was operated in splitless mode for 30 seconds, with the injector temperature set to 250 °C. The oven temperature program initiated at 50 °C (held for 5 minutes), then increased to 210 °C at a rate of 5 °C min^{-1}, followed by an increase to 300 °C at 20 °C/min (held for 1 minute). Helium served as the carrier gas, maintaining a constant flow of 1 mL/min. The MS source temperature

was set at 230 °C, while the quadrupole temperature was maintained at 150 °C. Positive electron ionization (EI+) was employed, with an electron energy level of 70 eV. The detector was operated in scan mode within the range of 35 to 500 m/z. Prior to measurements, the MS was tuned using perfluorotributylamine (PFTB). The GC and MS parameters were managed by Agilent Enhanced MSD ChemStation software, while Agilent MassHunter Workstation Qualitative Analysis B.08.00 software was utilized for chromatogram evaluation and comparison. Compound identification was conducted using the Agilent NIST 2017 Mass Spectral Library, supplemented by two additional libraries (W9N08 and W10N11) for verification. Kováts indices were calculated using the C8-C20 alkane calibration standard, and identification was further corroborated by comparing KI values obtained from the NIST webbook. Delta KIs were calculated by comparing the observed KIs with those from the NIST webbook database.

6.1.4 Statistical Analysis

The sum of (absolute) ranking differences (SRD) method, introduced by Héberger in 2010, serves as a framework for comparing methods/models to a predefined golden standard using rank numbers.

The initial validation of the SRD method was established by Héberger and Kollár-Hunek in 2011, termed the comparison of ranks with random numbers (CRRN). This approach generates an SRD distribution based on the number of rows within a dataset. The issue of repeated values (ties) was addressed in 2013, enabling the SRD to accommodate datasets containing ties (Kollár-Hunek and Héberger, 2013). Recent advancements in validation processes, including data splitting and resampling techniques, have been introduced by the developers (Héberger and Kollár-Hunek, 2019). The SRD method is freely accessible as a Microsoft Excel macro at http://aki.ttk.mta.hu/srd/ (accessed on 11 February 2021), as an R-Shiny online application at https://attilagere.shinyapps.io/srdonline/ (accessed on 11 February 2021), and as a Python implementation at https://github.com/davidbajusz/srdpy (accessed on 11 February 2021). The authors of the present study utilized the Microsoft Excel version. For each method four replicates were used.

6.2 Methodology Development in Electrophysiological Experiments

6.2.1 Insects Materials

Metcalfa pruinosa specimens were fieldcollected as first and second instar nymphs in Martonvásár, Hungary (47.318321, 18.780314) during July 2019. The collection was conducted from various deciduous plant species utilizing the beating method as described by (Schauff, 2001). A black beating sheet was placed beneath the host plants, and branches were sharply struck with

a stick. Specimens that fell onto the sheet were subsequently removed from the dark material using an aspirator. The collected specimens were transferred to potted boxwood (*Buxus sempervirens* L., Buxaceae) plants at the Júliannamajor Experimental Station of the Plant Protection Institute, Centre for Agricultural Research. The plants were covered with fine mesh and placed in climate chambers set at 26 ± 2 °C, with a relative humidity of 50 ± 10% and a photoperiod of L-D 16:8. Nymphs were allowed to develop on the same plants until they reached the adult stage, with fourth and fifth nymphal and adult stages used for the olfactory measurements.

6.2.2 Volatile Collection

Headspace volatiles were collected from shoots of tree of heaven (*Ailanthus altissima*), birthwort (*Aristolochia clematitis*), and flowering shoots of French marigold (*Tagetes patula*) in the early afternoon at room temperature in the laboratory. Cut branches were enclosed in a Nalophan sampling bag (Nalophan 471 mm, Kalle GmbH, Germany) up to 30 minutes post-cutting, allowing for 30 minutes of saturation of the airspace before sampling commenced. Volatile collections were conducted over a period of 4 hours with a 1 L/min airflow using an open-loop, dynamic volatile collection system equipped with a 5 mg active charcoal trap (Brechbühler AG, Schlieren, Switzerland). Subsequently, the adsorbed volatiles were eluted with 150 µL of dichloromethane and stored at -40 °C until chemical analysis or the electrophysiological studies (see below).

6.2.3 Analytical Measurements

The chemical composition of the plant volatiles was analysed using GC-MS with an HP Agilent 5890 GC and 5975 MS (Agilent Technologies, Palo Alto, USA) on an HP-5 UI capillary column (30 m × 0.25 mm × 0.25 µm, J&W Scientific, Folsom, CA, USA). Helium served as the carrier gas with a total column flow of 1 mL/min. A 1 µL sample was auto-injected into the split/splitless injection port of the GC, operated in splitless mode and heated to 270 °C with a 1-minute purge time. The oven temperature was initially held at 50 °C for 1 minute, then increased to 260 °C at a rate of 10 °C/min, where it was maintained for 10 minutes. The MS transfer line was heated to 250 °C. Positive electron ionization was employed with an electron energy level of 70 eV, and data were recorded at a rate of 2 scans/s in the range of 29-300 m/z.

The analysis and identification of physiologically active compounds from *T. patula* were subsequently validated through testing synthetic standards with GC-MS and GC-FID/EAD. High-purity synthetic volatile compounds, including (*Z*)-3-hexenol, 1-hexanol, *β*-myrcene, (*Z*)-3-hexenyl acetate, limonene, eucalyptol, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, linalool, methyl salicylate, piperitone, α-copaene, and *β*-caryophyllene, were procured from Sigma-Aldrich and diluted in

dichloromethane (HPLC grade, Merck). Additionally, DMNT was synthesized by Prof. Dr. Stephan Schulz.

The remaining 63 compounds were tentatively identified by matching their mass spectra with those in the MS libraries (NIST 17 and Wiley) using MassHunter (B.8.00) and by comparing calculated and published Kováts index values based on C8-C40 alkane calibration standards. The amounts of electrophysiologically active compounds were quantified using a three-point dose curve based on calibration with synthetic standards via GC-FID (see Table S3).

6.2.4 Electrophysiological Experiment

The sex of adult insects was determined by observing their genitalia under a stereomicroscope at ×10 magnification (Świerczewski et al., 2022). Tested juveniles were in the fourth or fifth nymphal stages. For the electrophysiological experiments, the heads of the insects were dissected with particular attention given to the removal of the labium. The flagellum was consistently trimmed, and the recording electrode filled with Ringer solution was positioned near the pedicel. The reference electrode was inserted into the head, penetrating the membrane between the head and the thorax.

The temperature program for GC-FID was identical to that used for GC-MS analysis. The antennal signal was converted to a digital signal using a high-input impedance DC amplifier interface (IDAC-2, Ockenfels Syntech GmbH). External amplification was set to 1 in the software. The antennal signal was recorded simultaneously with the FID signal using the GC-EAD 2012 software provided by the manufacturer (version 1.2.4, Syntech).

To assess the antennal sensitivity of *M. pruinosa*, GC-EAD recordings were completed using plant volatile extracts rather than a panel of synthetic standards, as the plant volatile extracts were considered ecologically more relevant. Both female and male adults were tested using *T. patula* (N_f = 9, N_m = 8), *A. altissima* (N_f = 3, N_m = 3), and *A. clematitis* (N_f = 4, N_m = 3) extracts. The sensitivity of nymphs was evaluated using *T. patula* (N_n = 9). Antennal responses were normalised according to the methods described by (Biasazin et al., 2019).

6.2.5 Statistical Analyses

Due to low sample sizes for *A. altissima* and *A. clematitis*, only qualitative assessments of electrophysiological responses were conducted for these species. Formal statistical analyses were performed exclusively on responses to *T. patula* volatile samples (see Table 1). To minimize noise and to focus on the presumably more important volatile components, we excluded components from the analyses if a response was undetectable in at least six individual antennal preparations (for details, see Table 1), resulting in the retention of 8 compounds for analysis.

We examined differences in responsiveness to compounds in the extracts between males and females, as well as between adults and nymphs. The number of responders per compound was analysed using Fisher's exact tests with the fisher_test function in the rstatix package (version 0.7.2) (Kassambara, 2020) in R (version 4.1.2, R Core Team, 2021). To mitigate the inflation of type I error rates due to multiple comparisons, we adjusted p-values using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

Table 1 *Successful replicates for electrophysiological responses of M. pruinosa.*

Compound	***Tagetes patula***			***Ailanthus altissima***		***Aristolochia clematis***	
	female	male	nymph	female	male	female	male
(*E*)-2-hexenal	4	4	4	3	3	4	3
(*E*)-*β*-ocimene	8	8	5	3	3	4	3
(*Z*)-3-hexenol acetate	6	4	5	3	3	4	2
(*Z*)-3-hexenol*	8	7	9	3	3	4	3
(*Z*)-*β*-ocimene*	8	8	9	-	-	4	3
1-hexanol*	7	7	9	3	3	1	2
2-ethylhexanol	3	3	8	-	-	-	-
bornyl acetate	-	-	-	-	-	3	3
camphene	-	-	-	-	-	1	3
camphor	-	-	-	-	-	4	3
cosmene	4	3	6	-	-	3	2
dendrolasin	4	3	5	-	-	-	-
DMNT*	9	8	9	3	3	-	-
eucalyptol	6	6	4	-	-	4	3
germacrene D	7	4	8	2	2	4	3
hexanal	-	-	-	3	2	-	-
lavender lactone	-	-	-	-	-	4	1
linalool	4	6	8	3	3	2	3
methyl salicylate*	9	7	9	3	3	-	-
myroxide*	8	8	9	-	-	-	-
nonanal	-	-	-	3	1	-	-
octanal	-	-	-	3	3	-	-
piperitone*	6	7	9	-	-	-	-
terpene_1	-	-	-	-	-	4	2
p-mentha-1,5-dien-8-ol	6	4	7	-	-	1	2
α-copaene	5	7	4	-	-	3	3
β-caryophyllene*	8	8	9	3	3	4	3
β-myrcene	3	3	3	-	-	3	3

*Included in formal statistical analysis.

To assess sensitivity across sexes and developmental stages, response amplitudes per compound were compared using the Wilcoxon rank-sum test, utilizing the wilcox_test function from the rstatix package. Again, p-values were reported after adjustment using the FDR method.

To analyze response amplitudes to the compounds present in *T. patula*, a linear mixed-effects model was implemented. Based on the outcomes of Fisher's exact tests and Wilcoxon rank-sum tests, data were pooled for adults and nymphs in this analysis. Amplitude was designated as

the response variable, with compound and sex treated as categorical fixed effects, and replicate number included as a random effect. To achieve a normal distribution of model residuals and ensure homogenity of variances, data were $\log_{10}$-transformed. Residual plots were examined to verify compliance with the assumptions of the tests. The interaction between sex and compound, along with the main effect of sex, were not significant and were thus removed from the model. The lmer function from the lme4 package (Bates et al., 2015) was utilized to fit the mixed-effects models. Post-hoc comparisons were conducted using the posthoc_Pairwise function from the grafify package (Shenoy, 2021) with p-values adjusted via the FDR method.

6.3 Development of Novel, Yeast-Based, Field-Effective Attractants for *D. suzukii*

6.3.1 Volatile Collection and Analytical Measurements

The headspace of yeast-containing lures was sampled using SPME both before placing the traps in the field and after three days of field incubation. A total volume of 10 mL was pipetted into a 50 mL sample tube and sealed with a cap lined with a PTFE septum. After 30 minutes of saturation, an SPME fiber (DVB/CAR/PDMS 50/30 μm, Supelco, Sigma-Aldrich) was inserted into the vial through the cap lined with the PTFE septum, and the adsorbent was exposed to the sample headspace for five minutes. Prior to each volatile sampling, the fibers were conditioned at 250 °C in the split/splitless injector of the GC-MS in split mode for ten minutes. After field incubation, three randomly selected lures from each treatment were pooled (to reach a total volume of 10 mL in the same 50 mL sample tubes). The volatile headspace of each pool was sampled using an SPME fiber as previously described. The SPME volatile samples were analysed via GC-MS (Agilent 5890 GC and 5975 MS, Agilent Technologies), equipped with an HP-5 UI capillary column (30 m × 0.25 mm × 0.25 μm, J&W). The system operated in splitless mode for 30 seconds, with the injector temperature set to 250 °C. The oven temperature was maintained at 40 °C for two minutes, then increased by 10 °C/min to 270 °C. Ionization voltage was set at 70 eV, scanning the mass-to-charge ratio (m/z) range of 29-300 with a rate of 2 scans/s, using helium as the mobile phase at a constant flow rate of 36 cm/s. Compounds were tentatively identified by matching their mass spectra against those in the MS libraries (NIST11 and Wiley) using ChemStation software (D.01.02.16). Identifications were corroborated by injection of synthetic references, and Kováts indices were calculated using C8-C20 alkane calibration standards, subsequently compared to those in the libraries.

6.3.2 Lure Preparation

Stock cultures of four yeast species – *Hanseniaspora uvarum* (HU; strain: Y.009147), *Metschnikowia pulcherrima* (MP; strain: Y.01700, E3/3), *Pichia terricola* (PT; strain: Y.00710),

and *Saccharomyces cerevisiae* (SC; ATCC 38976) – were obtained from the National Collection of Agricultural and Industrial Microorganisms at Institute of Food Science and Technology in Hungarian University of Agriculture and Life Sciences, Budapest. Each strain was originally isolated from the surfaces of stone fruits. Yeasts were maintained on MGYP plates (0.3 g/g% malt extract, 1.0 g/g% glucose, 0.3 g/g% yeast extract, and 2.0 g/g% agar) and re-streaked weekly.

To achieve high cell density for the lures, starter cultures were prepared from the stock cultures and incubated for 72 hours in 500 mL of autoclaved MGYP broth (Merck) using a horizontal shaker at 150 rpm and 26 °C. Cell density was estimated using Bürker chambers, and all liquid cultures were standardized to 129 ± 15.5 cells/mL in autoclaved MGYP broth. Additionally, *H. uvarum* cultures were diluted in autoclaved apple juice (AHU; Tesco PLC Ltd.) to compare the effects of fruit substrate with liquid broth. Apple juice was selected due to its similar color and transparency to that of the MGYP broth traps. A total of 200 mL of the lure cultures was dispensed into 500 mL transparent plastic bottle traps (perforated with 16 entry holes, approximately 3-4 mm in diameter, around the bottleneck). Control traps containing the same volume of apple cider vinegar (ACV) and non-inoculated broth were also prepared.

6.3.3 Field Work

The trapping experiment was conducted in an 11-hectare commercial sour cherry (*Prunus cerasus*) orchard (47°53′42.4″N, 19°04′43.0″E) in Berkenye, Hungary, from 22 September to 24 October 2019. Bottle traps – 500 mL plastic bottles with 9 3-4 mm diameter holes in the upper part – were suspended from the lower branches of the canopies (approximately 170 cm height), spaced 30 m apart in a triangular grid arrangement. Each treatment was replicated 10 times in a randomized manner. Lures were replaced, and catches were collected after three days. Temperature fluctuations in the experimental orchard were logged at three locations. Female and male *D. suzukii*, along with their seasonal morphs and other drosophilid species, were identified and counted.

6.3.4 Statistical Analysis

The number of *D. suzukii* adult specimens captured in each trap was counted, along with fve other identified *Drosophila* species. Catch data were analysed in R (v3.3.2, R Core Team, 2020). Generalized linear models (GLM) were fitted using the MASS package (v7.3-54; (Ripley et al., 2013). The data distribution was fitted to a Poisson model, with overdispersion assessed using the testDispersion function of the DHARMa package (v0.4.3;(Hartig and Hartig, 2017), comparing simulated data dispersion with observed data dispersion through non-parametric tests. In instances of significant overdispersion ($p < 0.05$), a negative binomial model was employed

instead of a Poisson model. Daily median temperature and trap positions were incorporated as model covariates.

Specificity for *D. suzukii* was defined as the proportion of *D. suzukii* catches relative to total catches, specificity for winter morphs as the proportion of winter morphs in the *D. suzukii* catches, and sex specificity as the proportion of females in the *D. suzukii* catches. A binomial general linear model was fitted to the proportion data.

Comparisons of lure catches were performed pairwise with Dunn-Šidák correction for multiple comparisons using the multcomp package (v1.4-16;(Hothorn et al., 2008).

The volatile data were normalised by dividing the peak area by the sum of peak areas for each sample. For data standardization, the mean area of each peak was subtracted from the peak area in the sample and divided by the standard deviation of the peak area across samples. To compare volatile profiles of yeast species, Jaccard dissimilarity indices were calculated on square-root transformed data, followed by NMDS and PERMANOVA analyses (with subsequent pairwise comparisons using Bonferroni correction for significance values) utilizing the vegan package (v2.5-7;(Oksanen et al., 2019). Results were visualized using the ggplot2 package (v3.3.5;(Wickham and Wickham, 2016).

7 RESULTS AND DISCUSSION

7.1 Comparison of Multiple Techniques for Sampling Optimization

7.1.1 *Volatile Compositions of* Lactuca sativa

A total of 149 volatile compounds were identified during the TIC analysis (Table S2). These compounds formed the basis for subsequent data analysis. The list of relevant lettuce volatiles was refined by focusing on those with an integrated area greater than 0.1% of the total integrated area and an identification match factor exceeding 80%. Compounds exhibiting ΔKIs greater than 10% were excluded from the list, except in cases where the match factor exceeded 90%.

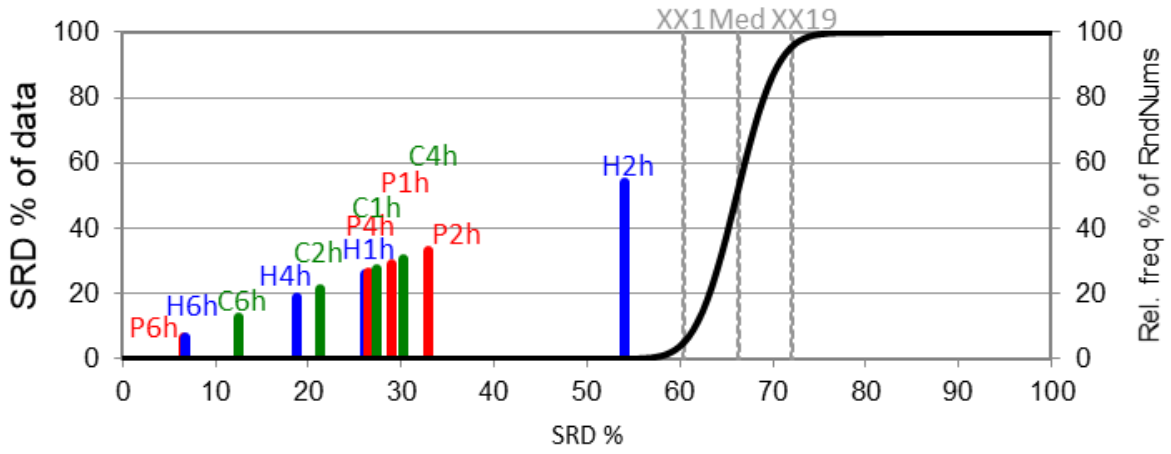

Figure 3 *The scaled sum of ranking differences values of the sampling procedure based on integrated peak area by sum of ranking differences. The maximum values of the compounds were used as the reference (benchmark) column. Scaled SRD values are plotted on the x-axis and left y-axis; the right y-axis shows the relative frequencies (black curve). Probability levels of 5% (XX1), median (Med), and 95% (XX19) are also given. Diagrams were produced by compound intensity values on a total ion chromatogram (N = 149). In the abbreviations, the letter denotes the type of adsorbent used (P for Porapak Q, H for Hayesep Q, and C for Carbotrap), while the number indicates the sampling duration (1, 2, 4, or 6 hours).*

Several of the identified compounds have been previously reported in other studies. For example, *α*- and *β*-pinene, D-limonene, and *β*-caryophyllene were identified in lettuce oil (Nomaani et al., 2013), while *γ*-elemene and D-limonene were detected in ready-to-use lettuce (Lonchamp et al., 2009). Additionally, *γ*-elemene, *β*-caryophyllene, and D-limonene were found in cut lettuce (Deza-Durand and Petersen, 2011). However, our study is distinctive in that it examines the volatile composition of the entire lettuce plant, providing a comprehensive list of volatile organic compounds.

7.1.2 Sampling Optimization Using Sum of Ranking Differences

A total of 149 compounds were identified during the evaluation of total ion chromatograms. Subsequently SRD analysis was performed on all compounds (N = 149), using

the compound intensity maxima as the reference column for the SRD. Among the various sampling intervals, the 6-hour sampling produced values most closely aligned with the reference values, indicating that this duration captured the highest amount of volatile compounds. Within the 6-hour sampling group, Porapak Q (P6h) and HayeSep Q (H6h) ranked highest, followed by the Carbotrap adsorbent (C6h) as shown in Figure 3.

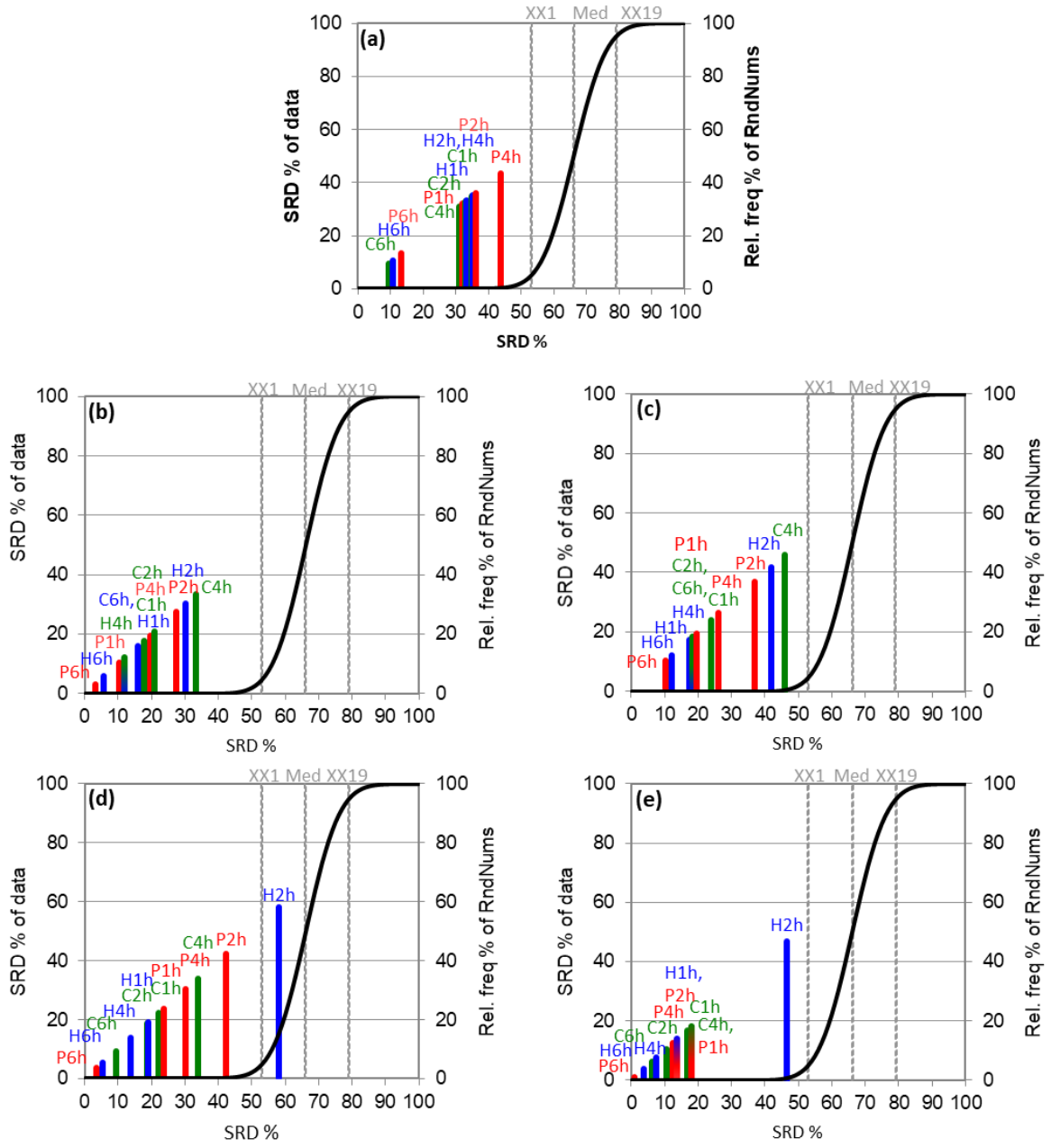

Figure 4 *The scaled sum of ranking differences values of the sampling procedure based on integrated peak area by sum of ranking differences. The maximum values of the compounds were used as the reference (benchmark) column. Scaled SRD values are plotted on the x-axis and left y-axis; the right y-axis shows the relative frequencies (black curve). Probability levels of 5% (XX1), median (Med), and 95% (XX19) are also given. In the abbreviations, the letter denotes the type of adsorbent used (P for Porapak Q, H for Hayesep Q, and C for Carbotrap), while the number indicates the sampling duration (1, 2, 4, or 6 hours). Diagrams were produced by volatility based on the elution order of total ion chromatogram: (a) the first 30 compounds between 0 and 30; compounds between (b) 30 and 60, (c) 60 and 90, and (d) 90 and 120; and (e) the last 29 compounds between 120 and 149.*

However, to optimize daily sampling efficiency, shorter sampling durations may be preferred. Notably, the 4-hour sampling with HayeSep Q (H4h) yielded satisfactory results, achieving an SRD value higher than 20%. Furthermore, shorter sampling times, such as the 2-hour sampling with Carbotrap (C2h) and the 1-hour sampling with HayeSep Q (H1h), proved to be more effective than the 4-hour sampling with Porapak Q (P4h). Thus, the H4h, C2h, and H1h methods were identified as both accurate and efficient for volatile collection, particularly when the primary research objective is to maximize the capture of volatiles from the headspace.

To assess the suitability of different adsorbents and sampling times for capturing volatile, semi-volatile, and less-volatile compounds, the dataset was split based on elution order (i.e., retention time). Generally, the more volatile a compound, the earlier it elutes in the total ion chromatogram – though the precise order depends on the column type. Five groups were formed, each containing 30 compounds, except for the last group, which contained 29 compounds. A separate SRD analysis was conducted for each group (Figure 4), allowing for the evaluation of adsorbent performance based on compound volatility.

For the most volatile compounds, all adsorbent types effectively captured them during the 6-hour sampling period (Figure 4a). Shorter sampling durations (2 or 4 hours) resulted in decreased compound intensities in the chromatogram, especially for more volatile compounds. Semi-volatile compounds (Figure 4bc) were also efficiently captured by the 6-hour sampling, but certain shorter sampling procedures, such as P1h, H4h (Figure 4bc), H1h, and C2h (Figure 4c), also showed acceptable performance, with SRD values below 20%. Among the 6-hour sampling procedures, P6h and H6h yielded results most similar to the reference – maximum compound intensity –, while P2h, H2h, and C4h were ranked lower.

In the case of low-volatility compounds (Figure 4de), the 6-hour sampling duration proved to be effective, with Porapak Q emerging as the top adsorbent. However, H4h and C2h also demonstrated satisfactory results, particularly when considering the desire to reduce sampling times. These two methods (H4h and C2h) were also among the top performers in the overall analysis of all compounds combined (Figure 3). For low-volatility compounds, the SRD value of P4h was approximately 15% (Figure 4e).

In conclusion, the 6-hour sampling period was found to be the most appropriate for capturing all volatile compounds, with Porapak Q being the most effective adsorbent overall. C4h was well-suited for collecting highly volatile compounds, while H4h, C2h, and H1h proved effective for semi-volatile and less-volatile compounds. However, a direct comparison with the trapping efficacy of single synthetic compounds would further improve the evaluation of these sampling procedures. Such an analysis could provide insights into potential biases in the

adsorption process and help refine the selection of sampling conditions for specific target compounds.

Consideration of SRD in the Context of Chemical Ecology

While the findings presented here provide a systematic evaluation of sampling methods and durations for maximizing volatile capture, it is important to contextualize these results within the specific demands of chemical ecology. In this field, it is not uncommon for certain minor compounds within a volatile profile to play significant roles in ecological interactions. This is particularly evident in the case of pheromones, where even trace amounts can elicit profound behavioural responses in insects (Arn et al., 1983). Similarly, specific groups of volatiles may hold greater importance depending on the ecological or behavioural context (Stensmyr et al., 2012).

Thus, the SRD method, which prioritizes comprehensive volatile collection, must be applied with careful consideration of the research objectives. While SRD provides a robust framework for ranking sampling methods based on compound intensity maxima, the biological relevance of individual compounds or compound groups must take precedence in chemical ecological studies. For example, a method with lower total compound capture may outperform others in a chemical ecological study if it selectively samples key behaviourally active compounds. Therefore, researchers should tailor the SRD analysis to the specific ecological questions being addressed, incorporating additional weighting or selection criteria to emphasize the importance of behaviourally or ecologically relevant compounds. This nuanced approach ensures that the methodology aligns with the unique demands of chemical ecology and strengthens the applicability of SRD in this specialized field.

7.2 Methodology Development in Electrophysiological Experiments

In our experiments, the fourth and fifth nymphal stages of *M. pruinosa* detected 20 volatile compounds from various chemical classes (Table 1, Figure 5). To our knowledge, only two previous studies have successfully conducted GC-EAD experiments on the nymphal stages of Auchenorrhyncha. In those studies, *L. delicatula* responded to two identified volatiles: carvone and linalool (Moon et al., 2011; Yoon et al., 2011). Out of the 77 volatile compounds we tested, 29 elicited physiological responses on the antennae of adult *M. pruinosa* (Figure 5, Table S3), including ketones, aldehydes, alcohols, terpenoids, and esters. Our findings suggest that *M. pruinosa* exhibits no clear preference for any specific chemical group (Figure 5).

In all GC-EAD measurements, a total of 29 volatile compounds elicited electrophysiological responses in adult *M. pruinosa* (Figure 5-7, Table 1). There were multiple common compounds in the headspaces, yet only certain species elicited responses to these compounds. For instance, camphene, present in the volatile profile of *A. clematis* (approximately

4 ng/µl) and *A. altissima* (below 1 ng/µl), was only active in the headspace of *A. clematis* (Figure 5 and 6). Similarly, 2-ethylhexanol was found in the headspaces of *T. patula* (ca. 23 ng/µl) and *A. altissima* (below 1 ng/µl), but responses were only triggered by the headspace of *T. patula* (Figure 5 and 7). Another compound, terpene_1, was detected in both *A. clematis* (ca. 64 ng/µl) and *T. patula* (ca. 2 ng/µl), yet a response was elicited only in the case of *A. clematis* (Figure 6 and 7).

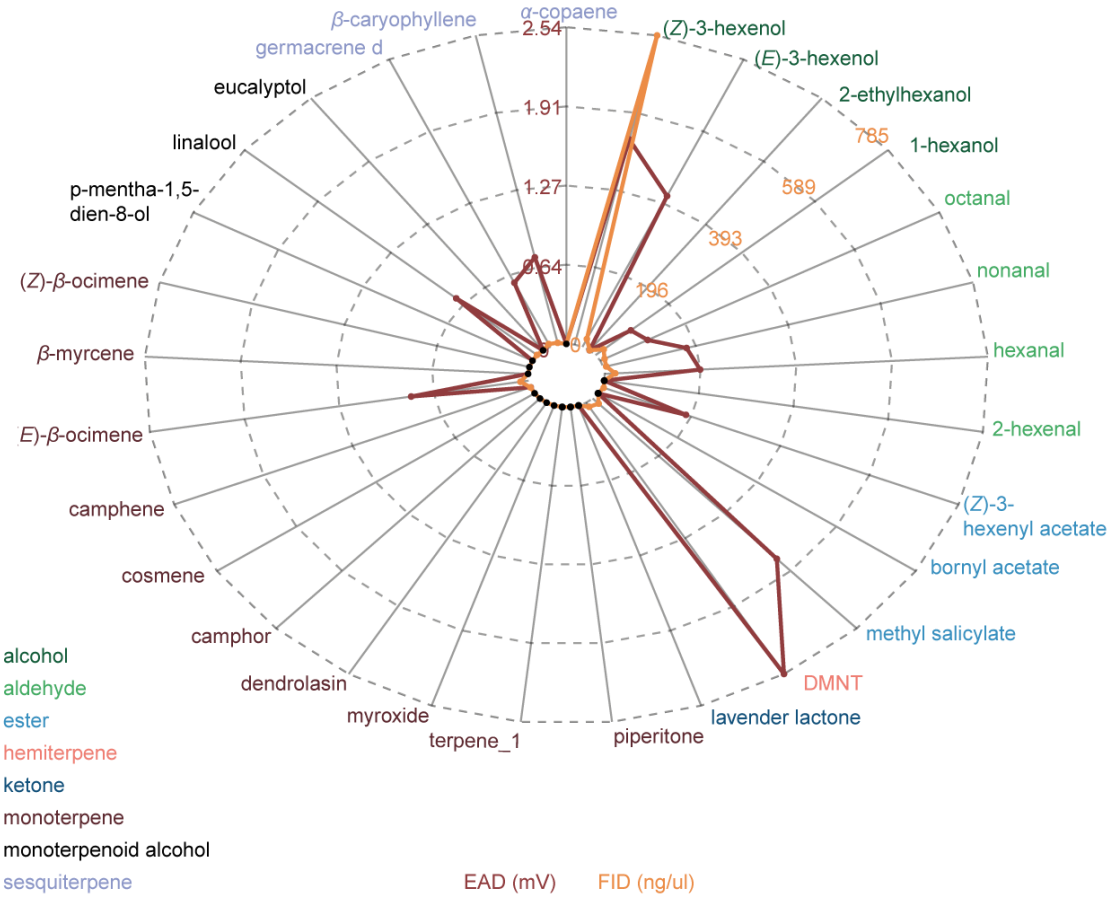

Figure 5 *Response amplitudes of* M. pruinosa *and amounts of electrophysiologically active compounds grouped by chemical classes. It depicts results obtained using the headspace volatile collections* Ailanthus altissima. *Black dots indicate the absence of the compound in the given headspace. Scale maxima are set to the same values for an effortless comparison.*

Similar research on *Philaenus spumarius* (Hemiptera: Aphrophoridae) and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) found that 65 out of 182 tested compounds evoked EAD responses in adult *P. spumarius*, while 16 compounds triggered responses in adult *N. campestris* (Anastasaki et al., 2021). In contrast, only one compound elicited an EAD response in both adult and nymphal *L. delicatula* out of four tested volatiles (Yoon et al., 2011), and 13 out of 50 compounds triggered responses in adult *Hyalesthes obsoletus* (Hemiptera: Cixiidae) (Riolo et al., 2012).

We speculate that the large number of response-eliciting compounds in *M. pruinosa* may be, at least in part, due to its extensive host plant range.

7.2.1 Evaluating Response Ratios and Amplitudes Across Sexes and Developmental Stages

No significant differences in the number of responders were found for any of the substances when comparing males to females or adults to nymphs (Table 2). Similarly, the response amplitudes were consistent across both sexes and developmental stages (Table 2).

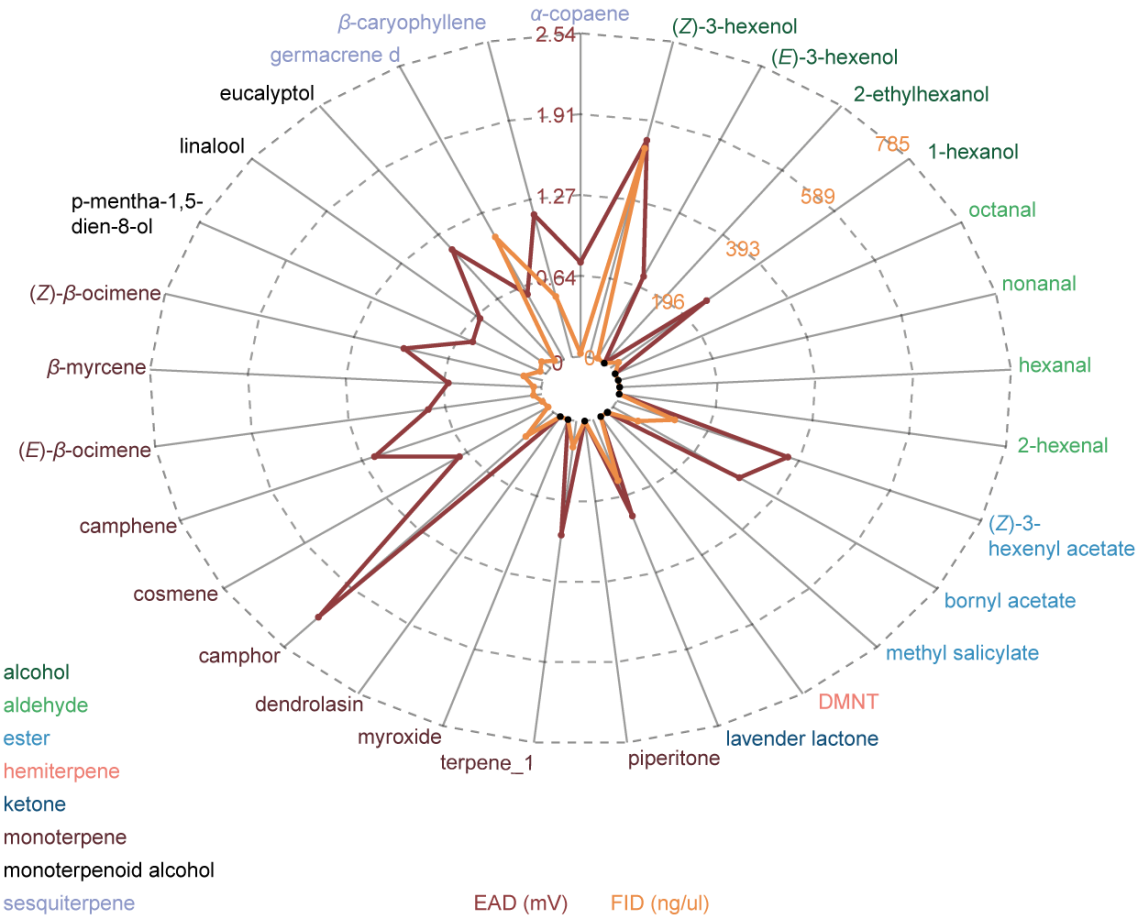

Figure 6 *Response amplitudes of* M. pruinosa *and amounts of electrophysiologically active compounds grouped by chemical classes. It depicts results obtained using the headspace volatile collections* Aristolochia clematis*. Black dots indicate the absence of the compound in the given headspace. Scale maxima are set to the same values for an effortless comparison.*

To determine which compounds induced the strongest antennal responses in adult *M. pruinosa*, the amplitudes of antennal responses were compared. The analysis revealed significant differences among the eight examined compounds ($F_{7,162} = 37.01$, $P < 0.001$) (Figure 8). DMNT, piperitone, and methyl salicylate elicited the strongest electrophysiological responses, despite the relatively low concentrations of DMNT and methyl salicylate in the headspace of *T. patula* (Table S3, Figure 7). Since the amplitude of electroantennographic responses correlates with the number

of activated sensilla on the antenna, and consequently with sensitivity to odours (Dekker et al., 2006; Spaethe et al., 2007; Jacob, 2018), these compounds may play critical roles in *M. pruinosa* host plant selection, potentially acting as attractants or repellents. *M. pruinosa* has a wide host plant range, which includes citrus plants, and shows a preference for grapefruit when grown adjacent to orange trees (Dean and Bailey, 1961). Notably, piperitone has been detected in the headspace of grapefruit but not in orange (Hong and Kim, 2016).

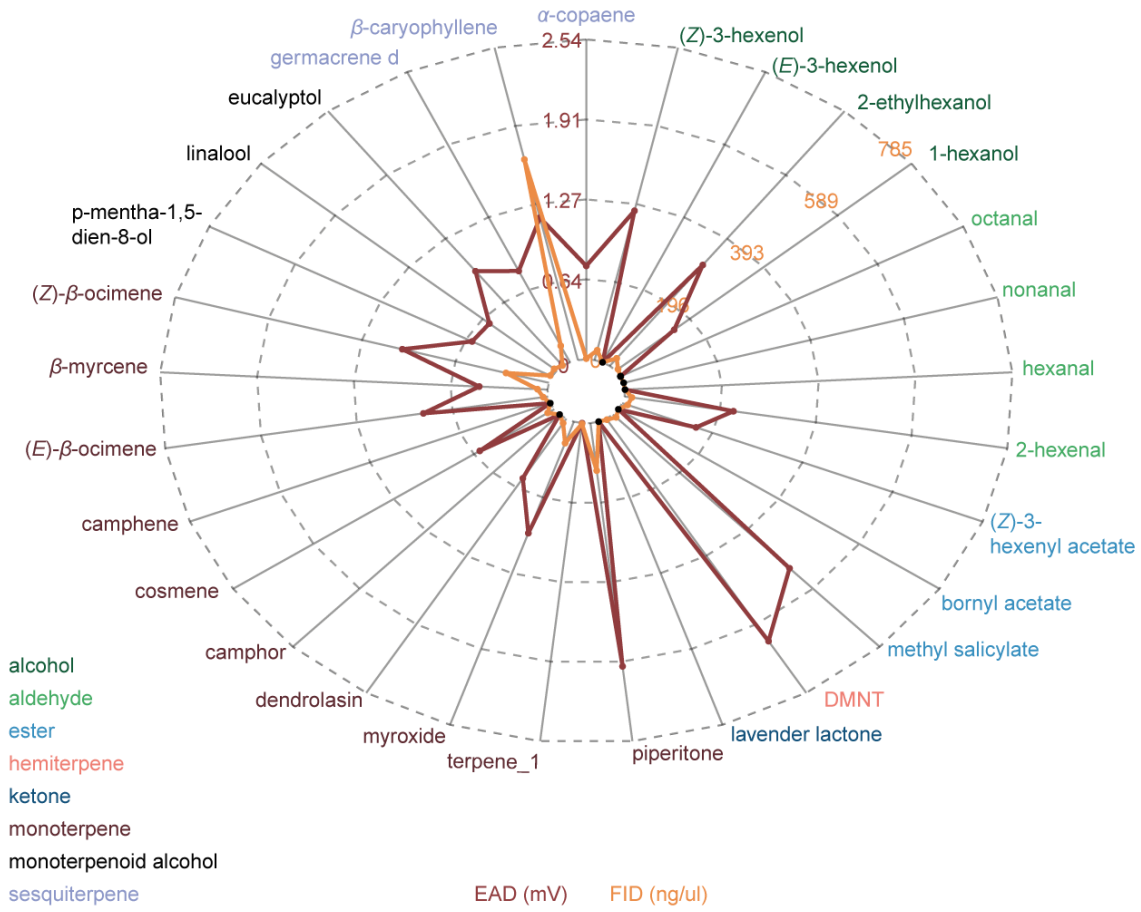

Figure 7 *Response amplitudes* M. pruinosa *and amounts of electrophysiologically active compounds grouped by chemical classes. It depicts results obtained using the headspace volatile collections from* Tagetes patula. *Black dots indicate the absence of the compound in the given headspace. Scale maxima are set to the same values for an effortless comparison.*

In contrast, responses to (*Z*)-3-hexenol were significantly weaker than those to methyl salicylate, and were similar to responses to (*Z*)-*β*-ocimene, *β*-caryophyllene, and myroxide. The weakest response was elicited by 1-hexanol. (*Z*)-3-hexenol, 1-hexanol, and (*Z*)-3-hexenyl acetate are green leaf volatiles commonly emitted by plants upon mechanical damage or herbivory (Matsui, 2006). As with many polyphagous herbivores, *M. pruinosa* may use the relative and absolute abundance of such ubiquitous plant volatiles to locate suitable habitats (Cha et al., 2011; Silva and Clarke, 2020).

We observed no differences in the number of responders or in response amplitudes between adult and nymph stages. This was surprising, considering that the number of sensilla on the

antennae increases throughout nymphal development in Hemipteran species (Keil, 1999; Wang et al., 2018). However, our results align with previous studies (Yoon et al., 2011; Moon et al., 2011), which reported that older nymphal stages perceive the same compounds as adult forms. This consistency in olfactory sensitivity across developmental stages may be due to olfactory plasticity occurring in the antennal lobe or higher brain centers, rather than in peripheral detection, as observed in other insects (Ignell et al., 2001; Gadenne et al., 2019).

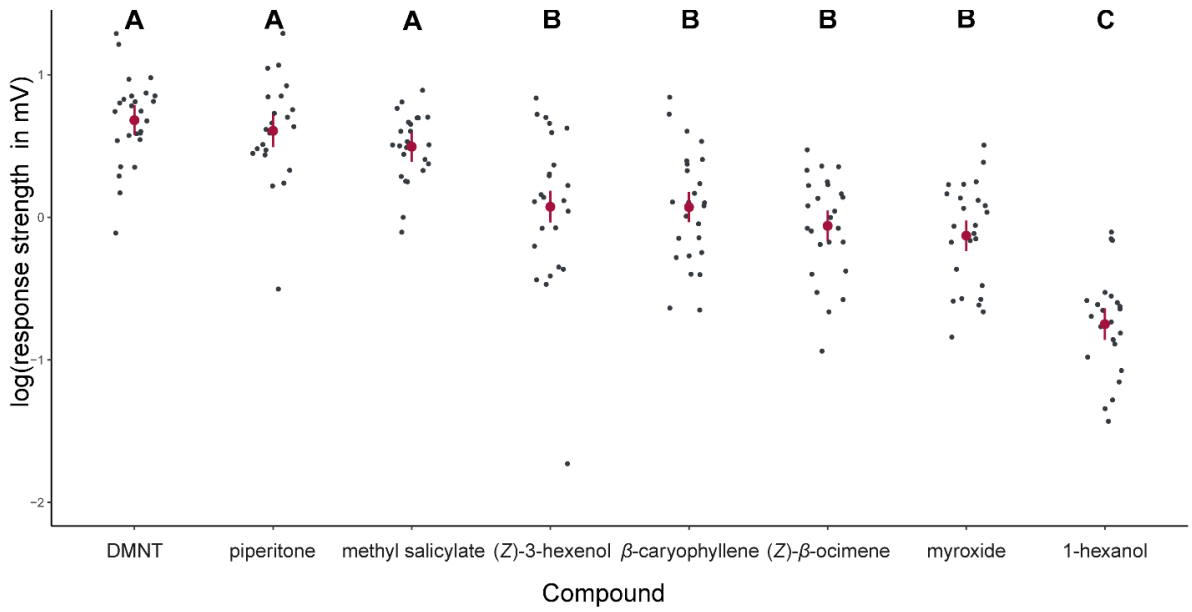

Figure 8 *Response amplitudes* M. pruinosa *to the analysed compounds present in the headspace of* T. patula. *Error bars represent estimated means and 84% confidence intervals (CI) calculated from linear mixed-effects models. Nonoverlapping CIs indicate significant differences between groups after correction for FDR. Letters above the error bars represent pairwise comparisons; groups not sharing any letter differ significantly ($p < 0.05$).*

Similarly, we found no qualitative or quantitative differences in peripheral sensitivity between adult males and females (Table 2). In contrast, in other Hemipteran species, such as the leafhopper *H. obsoletus*, only females responded to (*Z*)-3-hexenyl acetate and DMNT (Riolo et al., 2012). Additionally, female brown planthoppers (*Nilaparvata lugens*) were more responsive to (*Z*)-3-hexenol and linalool than males (Youn, 2002), while in *P. spumarius*, EAG amplitudes elicited by 1-octanol, eucalyptol, phenylacetaldehyde, and linalool significantly differed between sexes (Germinara et al., 2017). In species where olfactory sensitivity differs between sexes, it is likely that males and females occupy distinct ecological niches. In contrast, our findings suggest that both sexes of *M. pruinosa* use the same volatile cues for locating host plants for feeding, mating, and oviposition, as observed in other insects (Fein et al., 1982; Ukeh et al., 2010).

According to the "sequential cues hypothesis" proposed by Silva & Clarke (2020), polyphagous insects first locate potential host patches using commonly occurring plant volatiles. Once inside a patch, they employ additional cues – such as specific plant volatiles – to rank host

plants and select the most suitable ones. Nymphal stages of *M. pruinosa* may use common volatiles like (*Z*)-3-hexenol and 1-hexanol to locate habitats, and then refine their search based on volatiles associated with phloem-feeding damage – such as DMNT and methyl salicylate – along with visual and acoustic signals. Migrating adults may similarly use ubiquitous plant volatiles to search for suitable sites for mating and oviposition. However, without further behavioural experiments, we cannot definitively state whether these compounds play a significant role in host plant location and acceptance. A similar theory, termed the 'semiochemical-diversity hypothesis', was proposed by Zhang and Schlyter (2004) for bark beetles, suggesting that non-host volatiles can decrease the number of host trees subjected to mass attacks.

Table 2. *Results of comparing the sexes' (N = 17) and developmental stages' (N = 26) response ratio (Fisher's Exact test), and response amplitudes (Wilcoxon rank-sum test) of M. pruinosa using the headspace of* T. patula *volatile collection.*

		Fisher's Exact test				**Wilcoxon rank-sum test**				
	Compound	estimate	95% CI		p	estimate	W	95% CI		p
females vs. males	1-hexanol	0.520	0.007,	12.256	1	-0.166	13	-0.437,	0.053	0.99
	(*Z*)-3-hexenol	1.134	0.013,	100.694	1	0.419	42	-0.115,	0.943	0.847
	(*Z*)-*β*-ocimene	0.000	0.000,	43.846	1	-0.097	28	-0.465,	0.302	1
	β-caryophyllene	0.000	0.000,	43.846	1	0.127	38	-0.337,	0.620	1
	DMNT	0.000	0.000,	Inf	1	0.415	58	0.044,	0.994	0.288
	methyl salicylate	Inf	0.029,	Inf	1	0.088	34	-0.373,	0.377	1
	myroxide	0.000	0.000,	43.846	1	-0.135	27	-0.514,	0.225	1
	piperitone	0.307	0.005,	5.070	1	0.332	28	-0.662,	1.039	1
adults vs. nymphs	1-hexanol	0.00	0.00,	4.581	1	-0.015	61	-0.131,	0.203	1
	(*Z*)-3-hexenol	0.00	0.00,	10.201	1	-0.008	67	-0.683,	0.471	1
	(*Z*)-*β*-ocimene	0.00	0.00,	73.583	1	0.000	72	-0.291,	0.279	1
	β-caryophyllene	0.00	0.00,	73.583	1	-0.286	50	-0.593,	0.121	0.419
	DMNT	0.00	0.00,	Inf	1	0.475	111	-0.025,	0.998	0.265
	methyl salicylate	0.00	0.00,	73.583	1	-0.322	32	-0.630,	-0.029	0.185
	myroxide	0.00	0.00,	73.583	1	0.218	99	-0.034,	0.507	0.363
	piperitone	0.00	0.00,	2.777	1	-0.286	41	-0.968,	0.222	0.419

7.3 Development of Novel, Yeast-Based, Field-Effective Attractants for *D. suzukii*

7.3.1 Volatile Emissions of Yeast-Based Lures

Hanseniospora uvarum (HU) lures exhibited higher relative abundances of ethyl acetate, ethyl propionate, and butyl formate in the synthetic medium, a trend that persisted both before and after field placement (Figure 9).

Pichia terricola (PT) lures were the only ones to emit detectable amounts of ethyl octanoate, ethyl nonanoate, and ethyl decanoate prior to field placement, along with a significant relative abundance of ethyl hexanoate (Figure 9). Following field incubation, the emission of these ethyl esters remained dominant, although other compounds, such as ethyl butyrate, also became detectable (Figure 9).

When HU and PT were combined in equal amounts in the liquid medium (MIX), the resulting volatile emission profile resembled that of the PT lures, suggesting that PT outcompeted HU for growth in this medium (Figure 9).

The *Saccharomyces cerevisiae* (SC) lures emitted a higher ratio of 3-methyl butanol, 2-methyl butanol, and styrene, while exhibiting a lower ratio of other volatiles both before and after field incubation (Figure 9).

In contrast, when *H. uvarum* was grown in apple juice (AHU), the volatile emission profile was more diverse and significantly altered after field incubation. During this period, the ratios of (*E*)-2-hexenal and (*E*)-2-hexenyl acetate decreased, while the ratios of phenethyl acetate and other esters increased. After field incubation, the volatile profile of AHU lures showed elevated ratios of isoamyl acetate, isobutyl acetate, isoamyl propionate, and phenethyl acetate compared to the other lures (Figure 9).

7.3.2 Attractivity and Specificity for Spotted Wing Drosophila

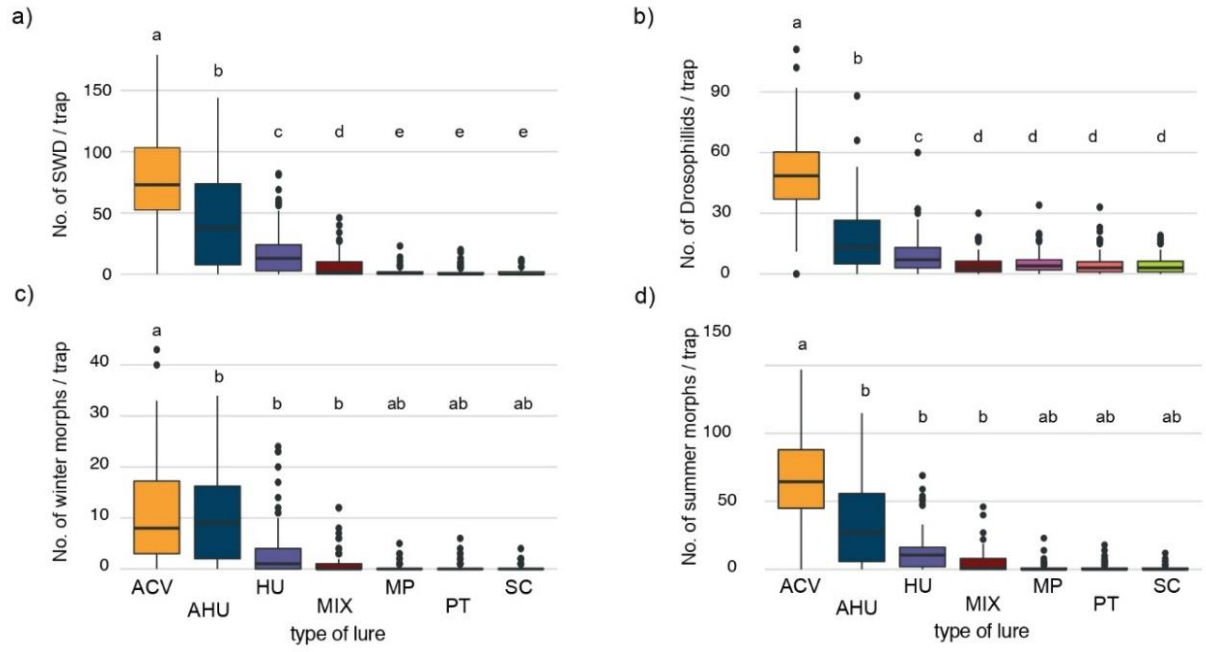

Figure 9 *The attractivity of different yeast lures and apple cider vinegar. The average catches of* Drosophila suzukii *(a), other Drosophilids (b), winter morphs (c) and summer morphs (d) per individual traps. Error bars show standard deviation. Treatments with different letters are significantly different based on generalized linear models and pairwise comparison of groups ($p < 0.05$). ACV, apple cider vinegar; AHU, apple juice inoculated with* Hanseniaspora uvarum*; HU,* Hanseniaspora uvarum*; MIX, mixture of* P. terricola *and* H. uvarum*; MP,* Metschnikowia pulcherrima*; PT,* Pichia terricola*; SC,* Saccharomyces cerevisiae.

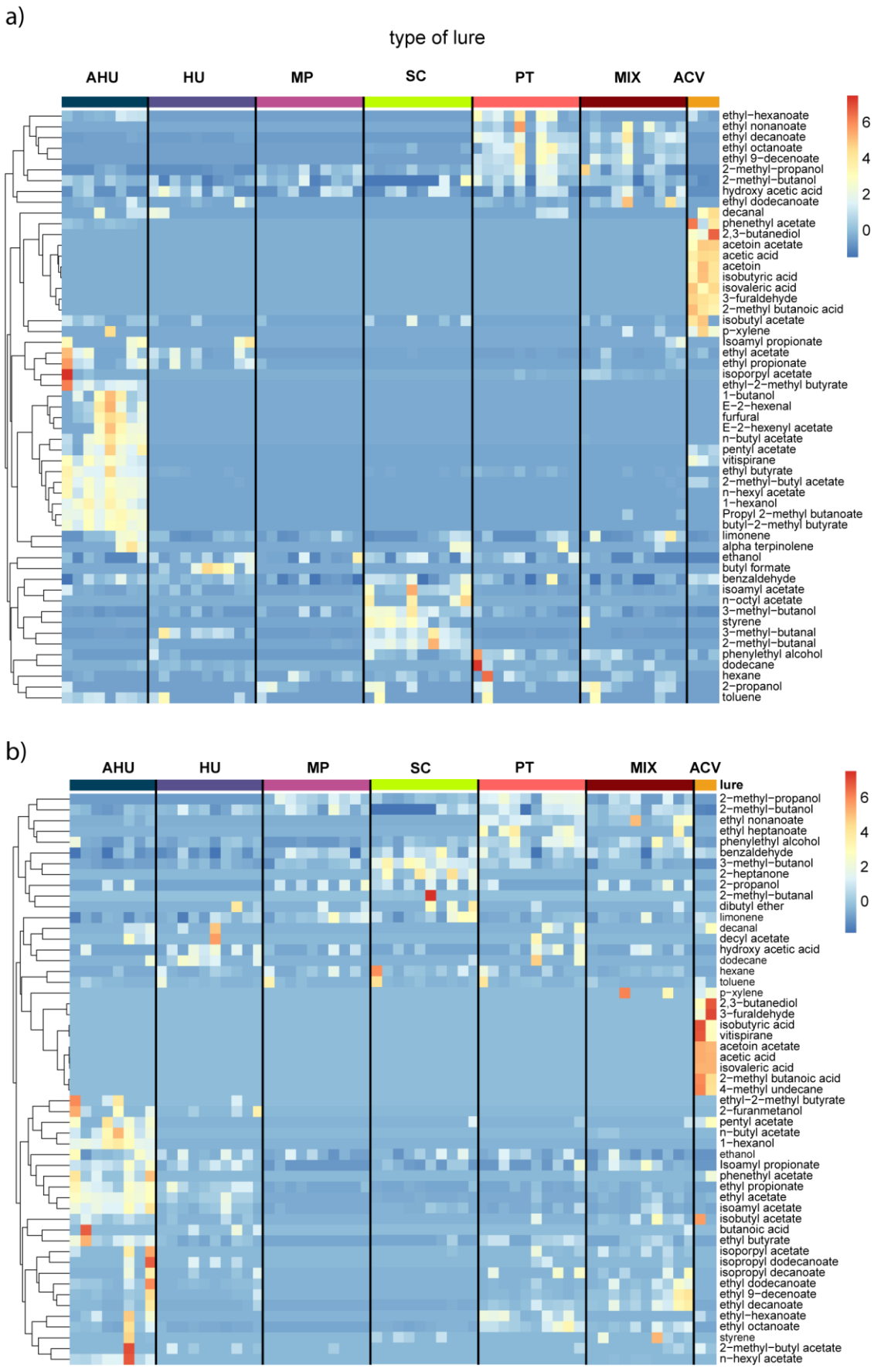

Figure 10 *Heatmap of volatile profiles of yeast-based lures (a) before and (a) field incubation. The colour code shows the standard score for each data point calculated from the relative area. AHU, apple juice with* Hanseniaspora uvarum, *HU,* H. uvarum, *MP,* Metschnikowia pulcherrima, *PT,* Pichia terricola, *SC,* Saccharomyces cerevisiae, *MIX, mixture of* P. terricola *and* H. uvarum *and apple cider vinegar (ACV).*

Table 3 *The statistical comparison of* Drosophila suzukii *and non-target drosophilid species. The table contains the medians and standard deviation of specimens caught per trap. Treatments with different superscript letters are significantly different based on generalized linear models and pairwise comparison of groups ($p < 0.05$). Model:negative binomial (NB) and binomial (B) distribution; Chi, chi-square value for factor treatment; P_{tr}, probability for the differences between treatments.*

	model	**Chi**	**P_{tr}**	**ACV**	**AHU**	**HU**	**PT**	**SC**	**MP**	**MIX**
D. suzukii (total)	NB	0.99	0.00	73 +/-39.1^{a}	37.5 +/-41.0^{b}	13 +/-19.6^{c}	0 +/-3.92^{e}	0 +/-2.27^{e}	0.5 +/-3.48^{e}	2 +/-9.73^{d}
D. suzukii (female)	NB	0.052	0.00	35 +/-21.1^{a}	20 +/-22.8^{a}	7 +/10.9^{b}	0 +/-2.11^{c}	0 +/-1.68^{c}	0 +/1.43^{c}	1.5 +/-5.04^{d}
D.melanogaster	NB	0.997	0.00	10.1 +/-5.5^{a}	4.11 +/-4.53^{b}	0.95 +/-1.67^{c}	0.162 +/-0.548^{d}	0.5 +/-0.219^{d}	0.15 +/-0.53^{d}	0.488 +/-0.955^{c}
D.immigrans	NB	1.000	0.00	7.2 +/-4.68^{a}	0.9 +/-1.51^{b}	0.312 +/-0.922^{c}	0.05 +/-0.219^{d}	0 +/-0^{d}	0.025 +/-0.157^{d}	0.0875 +/-0.326^{cd}
D.subobscura	NB	1.000	0.00	5.78 +/-3.8^{a}	1.35 +/-2.20^{b}	0.525 +/-1.10^{c}	0.0375 +/-0.335^{d}	0.0125 +/-0.112^{d}	0.0625 +/-0.291^{d}	0.0375 +/-0.191^{d}
Probability of *D. suzukii*	B	0.999	0.00	0.632 +/-0.01^{b}	0.745 +/-0.01^{a}	0.647 +/-0.02^{b}	0.252 +/-0.04^{c}	0.222 +/-0.04^{c}	0.222 +/-0.04^{c}	0.558 +/-0.03^{d}
Ratio of *D. suzukii* females	B	0.999	0.00	0.496 +/- 0.01^{a}	0.534 +/-0.03^{b}	0.561 +/-0.02^{b}	0.555 +/-0.08^{ab}	0.632 +/-0.09^{ab}	0.525 +/-0.08^{ab}	0.546 +/-0.04^{ab}
Ratio of winter morphs	B	1.000	0.00	0.136 +/-0.01^{a}	0.223 +/-0.01^{b}	0.206 +/-0.02^{b}	0.197 +/-0.06^{ab}	0.241 +/-0.08^{ab}	0.180 +/-0.070^{ab}	0.205 +/-0.04^{ab}
Ratio of winter females	B	0.999	0.00	0.511 +/0.03^{a}	0.577 +/-0.02^{ab}	0.663 +/-0.06^{b}	0.540 +/-0.2^{ab}	0.689 +/-0.2^{ab}	0.661 +/-0.2^{ab}	0.508 +/-0.1^{ab}

One critical criterion for environmentally friendly insect lures is their specificity to the target species, but we observed significant differences in attractivity and specificity among yeast-based lures for various vinegar fly species, which corresponded to differences in volatile emissions. Six vinegar fly species were identified in the trap samples, with *D. suzukii* being the most common. The main non-target catches were *D. melanogaster*, *D. immigrans*, and *D. subobscura*, while *D. obscura*, *D. tristis*, and *Phortica semivirgo* were captured sporadically (Table 3).

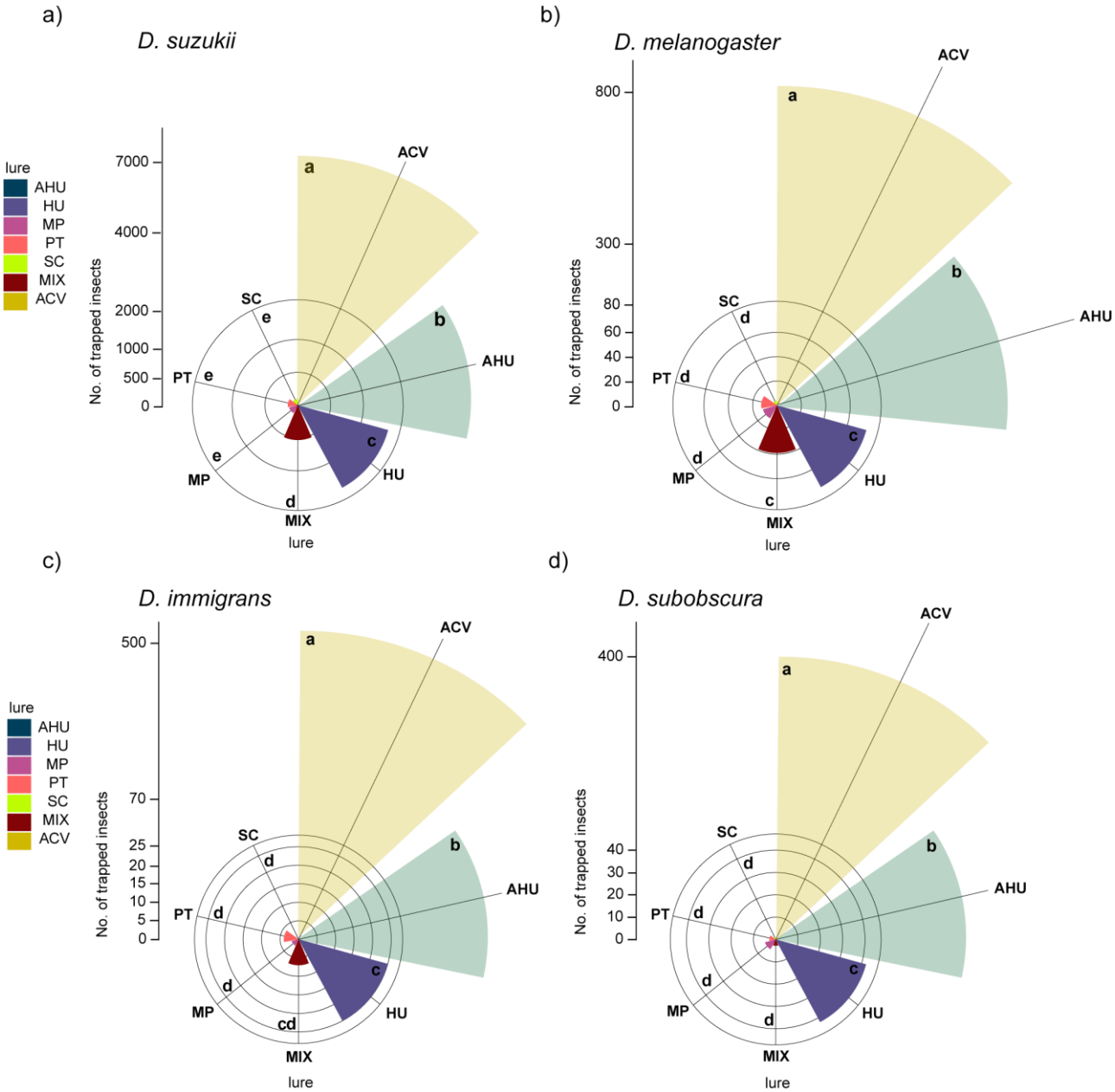

Figure 11 *The sum of target species (*D. suzukii*) and the identified non-target drosophilid specimens (*D. melanogaster, D. immigrans *and* D. subobscura*) caught during the field trials with apple cider vinegar (ACV) and traps baited with different yeast species: AHU, apple juice with* Hanseniaspora uvarum*; HU,* H. uvarum*; MIX, mixture of* Pichia terricola *and* H. uvarum*; MP,* Metschnikowia pulcherrima*; PT,* P. terricola*; SC,* Saccharomyces cerevisiae. *Traps with different superscript letters are significantly different based on generalized linear models and pairwise comparison of groups ($p < 0.05$).*

Pairwise comparisons indicated that AHU had the highest specificity for *D. suzukii* ($p = 0.001$), followed by ACV, HU, and MIX, though these were less specific than AHU. Both ACV and AHU caught the most *D. suzukii* females, with no significant difference between them ($p = 0.001$). The HU lure was less attractive than AHU and ACV but more effective than other yeasts (Table 3).

Apple cider vinegar (ACV) was less specific for *D. suzukii*, but attracted a greater overall number of drosophilids compared to other yeast lures (Figures 9 and 10, Table 3). We hypothesize that the presence of volatile carboxylic acids, such as acetic acid, in the ACV headspace – and their absence in yeast-based lures – may account for this difference. Several vinegar fly species, including *D. melanogaster*, *D. simulans*, and *D. suzukii*, host acetic acid bacteria from the genera *Acetobacter*, *Gluconobacter*, and *Komagataeibacter* (Chandler et al., 2011; Mazzetto et al., 2016). These bacteria provide fitness benefits for *D. melanogaster* and some species increase the flies' lifespan, though not female fecundity (Gould et al., 2018). *D. suzukii* is attracted to volatile metabolites of these bacteria, such as acetic acid, isobutyric acid, and 2-methylbutyric acid (Mazzetto et al., 2016), which were emitted only from ACV lures in this study.

In our experiments, *H. uvarum* in synthetic medium attracted more *D. suzukii* than other yeasts in the same medium (Figures 9 and 10, Table 3). The likelihood of catching female *D. suzukii* was highest with AHU and HU, and significantly lower with ACV and other yeast lures ($p = 0.001$, Table 3). This result aligns with earlier field studies where *H. uvarum* baits were more attractive to *D. suzukii* adults than *P. terricola* and *H. opuntiae* baits (Bueno et al., 2020) and more specific than *M. pulcherrima* and *S. cerevisiae* (Jones et al., 2021). In this study, *H. uvarum* cultures emitted higher ratios of ethyl acetate, ethyl propionate, and isoamyl acetate than other tested lures, and both isoamyl acetate and ethyl acetate are known attractants for mated *D. suzukii* females (Revadi et al., 2013; Piñero et al., 2019). However, in some studies, isoamyl acetate has been shown to be irrelevant in attracting *D. suzukii* to acetic acid and ethanol (Cha et al., 2012).

The association between *H. uvarum* and *D. suzukii* is not exclusive, as other drosophilids, including *D. melanogaster* and *D. immigrans*, are also attracted to *H. uvarum* (Palanca et al., 2013; Bates et al., 2015; Günther and Goddard, 2019). This was confirmed by our results, which showed that *D. melanogaster*, *D. subobscura*, and *D. immigrans* were significantly more abundant in *H. uvarum*-baited traps than in other yeast-baited traps (Figure 8, Table 3). Differences in fitness benefits provided by specific yeast diets for *D. suzukii*, such as faster development and higher fertility, have not always been conclusive (Bellutti et al., 2018; Murgier et al., 2019). Yeasts, especially *H. uvarum*, are associated with larval and adult food sources and offer fitness benefits to *D. suzukii* females (Spitaler et al., 2020). *H. uvarum* diets were found to shorten egg-to-pupa development, while egg-to-adult development was significantly shorter on *S. cerevisiae* diets

(Murgier et al., 2019). The yeast-vinegar fly association may not only result from natural selection but also from random encounters with fruit epiphytic yeasts (Bing et al., 2018; Jiménez-Padilla et al., 2020; Jones et al., 2021).

The growth substrate also influenced the attractivity of *H. uvarum* baits, with *H. uvarum*-inoculated apple juice attracting more *D. suzukii* than *H. uvarum* in synthetic medium. The volatile analysis showed a diverse blend of fermentation volatiles from apple juice baits, including isoamyl acetate, isobutyl acetate, isoamyl propionate, and 2-phenethyl acetate, which were potential attractants emitted at higher relative ratios than from other lures (Figure 9). Interestingly, *H. uvarum* in both apple juice and synthetic media attracted female *D. suzukii* more effectively than ACV.

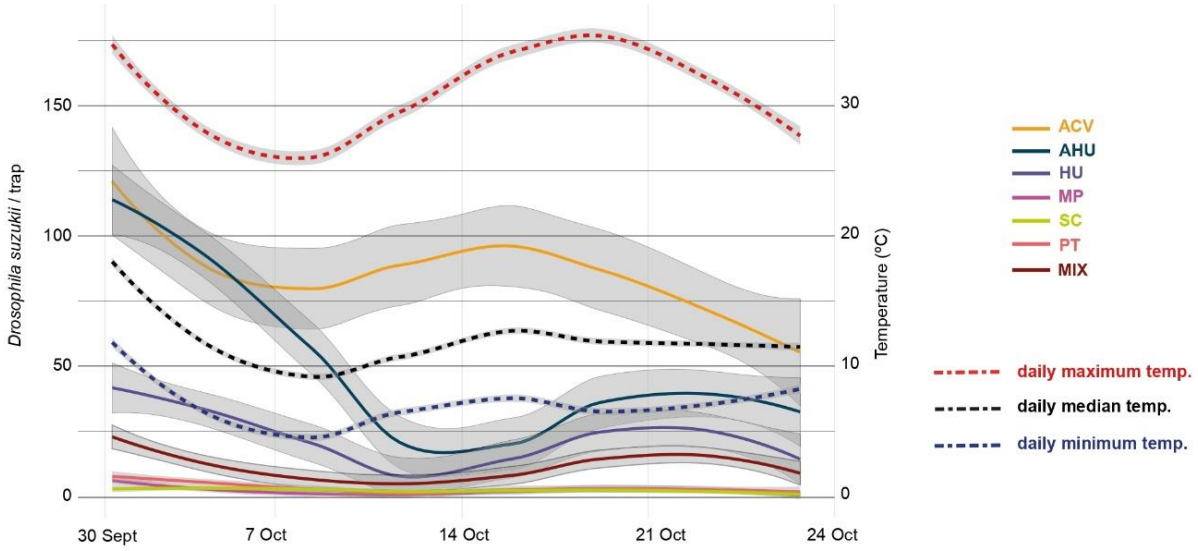

Figure 12 *The average of* D. suzukii *catches of different lures plotted against the daily minimum, maximum and median temperature during the field trial. ACV, apple cider vinegar; AHU, apple juice inoculated with* Hanseniaspora uvarum*; HU,* H. uvarum*; MIX, mixture of* P. terricola *and* H. uvarum*; MP,* Metschnikowia pulcherrima*; PT,* Pichia terricola*; SC,* Saccharomyces cerevisiae.

Generalized linear model (GLM) analysis revealed a significant interaction between *Drosophila suzukii* catches and daily minimum and maximum temperatures for all lures. Fluctuations in the number of trapped animals can be attributed to both reduced volatility (Polizzi et al., 2011; Misztal et al., 2018) and decreased flight activity (De Araujo et al., 2019) at lower temperatures. Yeast lure efficiency declined in the third week of the trial during the highest recorded temperatures, while ACV showed the opposite trend (Figure 11). Although environmental factors and yeast growth media can modulate yeast volatile emissions (Hamby et al., 2012; Lasa et al., 2019), our results support the stability of the *H. uvarum* – *D. suzukii* association.

ACV and AHU captured the highest number of winter-morph *D. suzukii*, with *Hanseniospora*-baited traps outperforming the other liquid-medium lures ($p = 0.001$; Figures 9c). Yeast-based lures were more specific for winter morphs than ACV, and yeast lures did not differ from each other in specificity for these morphs. Although ACV and AHU showed similar effectiveness in catching winter-morph females, HU had a higher probability of catching them compared to AHU and ACV (Table 3). Other yeast lures did not differ significantly in their probability of winter-morph female catches (Table 3). The winter-morph females of *D. suzukii*, which have different preferences, are difficult to study due to their lower activity and unknown overwintering sites. Our results suggest that *H. uvarum* in synthetic medium attracts winter-morph females more effectively than *H. uvarum*-inoculated apple juice or ACV (Figures 9 and 10). Previous findings showed that females collected from ripe fruit surfaces or fruit-based lures are more likely to have mature eggs in their ovaries than those collected from fermentation traps (Swoboda-Bhattarai et al., 2017; Wong et al., 2018). Mating status also influences OR expression in the antennae of female *D. suzukii* (Crava et al., 2019).

Our study indicates that winter-morph females with arrested ovary development are more attracted to yeast volatiles in the absence of fruit volatile backgrounds. Moreover, the reduced sensitivity of winter-morph female antennae to acetic acid and isoamyl acetate (Kirkpatrick et al., 2018) could explain their lower attraction to common fruit fermentation traps.

Given the metabolic diversity of yeasts, there is potential to design more attractive and specific baits for monitoring and mass trapping. Our findings suggest that *H. uvarum* is more attractive to *D. suzukii*, especially when paired with fruit substrates, and that yeast-fermented fruit substrates are more specific for females than ACV-based traps. However, caution is needed when using traditional fermentation baits, as they may not accurately represent adult fly populations.

8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Comparison of Multiple Techniques for Sampling Optimization

The SRD method was employed for the first time to assess various sampling procedures, yielding robust and validated results. The analysis identified 6-hour sampling intervals as the optimal procedures. However, for those aiming to decrease sampling time and increase throughput, alternatives such as H4h, C2h are also feasible options. As anticipated, the greatest differentiation among the sampling procedures was achieved by including all 149 identified compounds in the SRD analysis. Notably, the rankings produced by SRD showed only minor variations when considering just the top 20 most intense compounds. This finding indicates that focusing solely on these 20 compounds may be sufficient to determine an optimal sampling procedure. Given its non-parametric nature, SRD can be applied to assess any sampling technique, including various volatile collection traps, stir-bar sorptive extraction, headspace sorptive extraction, solid-phase microextraction, and dynamic headspace systems. By categorizing compounds into groups (such as aldehydes, ketones, terpenes, etc.), SRD can rank them according to the sampling procedures, potentially revealing which compound types are most effectively adsorbed by each method.

Due to the variations in the retention and loss of different types of volatile compounds – dependent on both the adsorbent used and the elution method – pure headspace injection stands out as the most reliable approach for accurate headspace analysis. This method minimizes the loss of volatiles during sampling and eluting, ensuring a more comprehensive representation of the compound profile. However, it should be noted that mass spectrometers may not always provide sufficient sensitivity for detecting low-concentration volatiles, which could impact the overall analysis precision of this method. Therefore, future advancements in detection sensitivity, along with continued evaluation of alternative sampling techniques, will be crucial for optimizing volatile compound analysis.

8.2 Methodology Development in Electrophysiological Experiments

In our study, we identified 29 volatile compounds that elicited electrophysiological responses in adult *M. pruinosa*, reflecting the insect's sensitivity to a wide range of chemical cues, with no clear preference for specific compound classes. Our findings align with previous research on similar insect species, though the broad range of response-eliciting compounds in *M. pruinosa* likely reflects its extensive host plant range. Interestingly, no significant differences were observed in response amplitudes or the number of responders between males and females, or across developmental stages, indicating that both sexes and nymphal stages use the same volatile cues for locating feeding and oviposition sites. These results suggest that *M. pruinosa*, like other

polyphagous herbivores, relies on common plant volatiles such as (*Z*)-3-hexenol and 1-hexanol to locate suitable habitats, while more specific volatiles like DMNT and methyl salicylate may play a crucial role in host plant selection. However, additional behavioral studies are required to confirm the ecological significance of these volatiles in *M. pruinosa*'s host plant location and acceptance strategies.

Future research should prioritize combining behavioral assays with electrophysiological data to clarify the role of specific volatile compounds in *M. pruinosa*'s host plant selection. Field experiments are particularly crucial for testing whether compounds such as DMNT and methyl salicylate function as attractants or repellents under natural conditions, where multiple sensory cues interact. Additionally, expanding the number of tested compounds and using advanced techniques such as olfactometers in both laboratory and field settings could enhance our understanding of volatile-mediated host selection.

8.3 Development of Novel, Yeast-Based, Field-Effective Attractants for *D. suzukii*

Our study confirmed that yeast-based lures, particularly those inoculated with *Hanseniaspora uvarum*, emitted diverse volatile profiles that influenced their attractiveness to *D. suzukii*. *H. uvarum*-inoculated apple juice outperformed other yeast lures in attracting *D. suzukii*, demonstrating a high specificity for *D. suzukii*, especially for females and winter morphs. While apple cider vinegar traps generally caught more drosophilid species, the yeast-based lures – especially *H. uvarum*-inoculated apple juice – were more specific for *D. suzukii*. This specificity likely resulted from the volatile profiles of the yeast cultures, particularly esters like isoamyl acetate and ethyl acetate, known to attract *D. suzukii* females. Our results underscore the importance of volatile composition in bait design and highlight the significant role of *H. uvarum* in attracting both *D. suzukii* and non-target drosophilid species. These findings align with previous research, supporting the hypothesis that *H. uvarum* can provide fitness benefits for *D. suzukii* and its close association with *D. suzukii* in natural and agricultural environments.

Future research should focus on combining behavioural assays with field experiments to better understand the olfactory responses of *D. suzukii* to yeast-based lures. Field experiments are essential to evaluate the long-term effectiveness of these lures in varying environmental conditions and to optimize their specificity for *D. suzukii* while minimizing non-target captures. Additionally, investigating the attractivity of different yeast strains under natural conditions, combined with behavioural observations, could lead to the development of more precise and efficient monitoring and trapping tools. Special attention should be given to the volatile blends emitted by *H. uvarum*-fermented fruit substrates, as they may offer more species-specific and environmentally friendly alternatives for control and monitor *D. suzukii*. In future studies, the reporting of non-target species

should be considered a fundamental practice, even if it requires more time and effort. Including such data is crucial for a comprehensive assessment of the environmental particularly in terms of biodiversity conservation. By documenting non-target species, researchers can provide valuable insights into the broader ecological consequences helping to develop more sustainable and environmentally responsible solutions.

9 NEW SCIENTIFIC RESULTS

The results of this study present several novel scientific findings in the field of volatile compound analysis and sampling optimization.

- First, the application of the SRD method to evaluate and optimize sampling procedures for volatile, semi-volatile, and low-volatile compounds marks a significant advancement. Notably, SRD has not been applied to such a broad range of sampling procedures before, making this a novel use of the method. An important discovery is that using a reduced number of compounds – specifically the top 20 most intensive – can still effectively determine optimal sampling procedures without sacrificing accuracy. (*This part of the thesis was published as: Radványi, D., Szelényi, M., Gere, A., & Molnár, B. P. (2021). From Sampling to Analysis: How to Achieve the Best Sample Throughput via Sampling Optimization and Relevant Compound Analysis Using Sum of Ranking Differences Method?. Foods (2021: Q1; IP: 5.3), 10(11), 2681.*)
- Second, we developed a new method for successful electrophysiological mesurements with the hemipteran species, *M. pruinosa*. For the first time, 29 volatile compounds were found to elicit electrophysiological responses in both adult and nymphal stages of *M. pruinosa*. These compounds span various chemical classes, such as terpenoids, ketones, aldehydes, alcohols, and esters, showcasing *M. pruinosa*'s broad olfactory detection capabilities. Interestingly, there were no observed qualitative or quantitative differences in olfactory sensitivity between males and females, or between adults and nymphs, suggesting that *M. pruinosa* relies on similar volatile cues throughout its life cycle, likely due to overlapping ecological roles. (*This part of the thesis was published as: Szelényi, M. O., Erdei, A. L., Molnár, B. P., & Tholt, G. (2024). Antennal olfactory sensitivity and its age-dependence in the hemimetabolous insect* Metcalfa pruinosa*. Journal of Applied Entomology (2024: Q2; IF: 1.9). Volume148, Issue4 May 2024 Pages 424-433.*)
- Third, our findings indicate that while traditional fermentation baits like apple cider vinegar are effective in capturing a broad range of drosophilids, yeast-based lures – particularly those inoculated with *Hanseniaspora uvarum* – provide enhanced specificity for *Drosophila suzukii*. These yeast-based lures proved valuable for monitoring and trapping *D. suzukii* across different morphs. Furthermore, our study also highlighted the presence of a wide variety of non-target drosophilids, emphasizing the need to account for both target and non-target species when designing pest monitoring tools. (*This part of the thesis was published as: Erdei, A. L., Szelényi, M. O., Deutsch, F., Rikk, P., & Molnár, B. P. (2022). Lure design for* Drosophila suzukii *based on liquid culture of fruit epiphytic*

yeasts: Comparing the attractivity of fermentation volatiles for seasonal morphs. Journal of Applied Entomology (2022: Q2; IP: 2.0), 146(6), 773-785.)

These findings offer practical insights for optimizing volatile sampling and advancing pest management strategies, particularly for species such as *D. suzukii* and *M. pruinosa*.

10 SUMMARY

Chemical ecology is essential for understanding the intricate interactions between organisms and the chemicals present in their environment. Its significance goes beyond basic biological research, directly influencing agriculture and plant protection. The need to apply chemical ecological principles in plant protection has become more urgent due to the rise of invasive species, which threaten ecosystems and agricultural systems alike. In pest management, insights into how insects respond to plant-derived chemicals allow for the development of more sustainable and targeted control strategies, which can minimize reliance on broad-spectrum chemical pesticides that often harm the environment. This makes chemical ecology increasingly vital to modern agriculture, not only protecting crops but also promoting food security and reducing negative impacts on biodiversity.

Different sampling methods can significantly impact the characterization of volatile organic compounds, which in turn may influence the interpretation and outcomes of the results from electrophysiological and trapping experiments. Therefore, optimizing the sampling process is crucial to ensure accurate VOC analysis. In our study, we tested three different adsorbents and four sampling time intervals, using a non-parametric statistical method called Sum of Ranking Differences. One key finding was that even when using a reduced set of compounds, it is still possible to determine the optimal sampling procedure. Moreover, by categorizing compounds into chemical groups, SRD can rank the sampling procedures, potentially revealing which types of compounds are most effectively adsorbed by each method.

The EAG response amplitude depends on the receptor potential of individual ORNs and the density of responsive ORNs near the electrode, especially in drosophilid-like antennae. Proper antennal preparation for EAG recordings should be species-specific, accounting for antennal shape and structure. Notably, this study marks the first successful electroantennographical recordings conducted with *M. pruinosa* both adult and nymph stages. These detected compounds span a diverse range of chemical classes, illustrating *M. pruinosa*'s broad olfactory detection capabilities. Interestingly, no significant differences were found in olfactory sensitivity between males and females or between life stages, indicating that *M. pruinosa* likely relies on similar volatile cues throughout its lifecycle, potentially due to their overlapping ecological roles. Future research should focus on behavioural assays. Field experiments will be also essential for testing these compounds under natural conditions, where multiple sensory cues are at play. Additionally, expanding the range of compounds tested could provide deeper insights into volatile-mediated host selection, ultimately aiding the development of more effective pest management strategies.

One critical criterion for environmentally friendly insect lures is their specificity to the target species. Our study confirmed that while apple cider vinegar traps generally caught more drosophilid species, the yeast-based lures – especially *H. uvarum*-inoculated apple juice – were more specific for *D. suzukii*. This specificity likely resulted from the volatile profiles of the yeast cultures. Our results underscore the importance of volatile composition in bait design and highlight the significant role of associations between yeasts and insects. In future studies, the reporting of non-target species should be considered a fundamental practice, even if it requires more time and effort. Including such data is crucial for a comprehensive assessment of the environmental particularly in terms of biodiversity conservation.

In conclusion, this thesis has followed the traditional workflow of chemical ecology to explore multifaceted strategies for pest management, with a particular focus on invasive species. By conducting detailed studies at different stages of the chemical ecology process – from the collection and analysis of volatile organic compounds to electrophysiological measurements and field trapping experiments – I aimed to provide both immediate and long-term solutions for effective pest control.

The studies on *Metcalfa pruinosa* highlight the role of chemical ecology in understanding sensory perception mechanisms, while those on *Drosophila suzukii* demonstrate its importance in deciphering insect behaviour, aiding the development of targeted management strategies. These species, which pose significant threats to agriculture, have been shown to respond to specific chemical cues that can be exploited for environmentally sustainable control methods. By employing innovative approaches, such as the optimization of sampling procedures and the creation of more selective lures, my research offers valuable insights into the application of chemical ecology for crop protection and biodiversity conservation.

The broader implications of this work reinforce the argument made in the introduction: chemical ecology is not only foundational to biological research but also critical for addressing the pressing challenges of modern agriculture. As the spread of invasive species accelerates, the need for adaptive and environmentally responsible pest management becomes increasingly urgent. The findings of this thesis contribute to this goal by providing practical tools and strategies that can be integrated into future pest management practices, while also laying the groundwork for further research into chemical interactions and their role in sustaining healthy ecosystems.

11 ÖSSZEFOGLALÁS

A kémiai ökológia kulcsfontosságú az élőlények közötti bonyolult kölcsönhatások és a környezetükben jelenlévő kémiai anyagok megértésében. Jelentősége túlmutat az alapvető biológiai kutatásokon, közvetlen hatása van a mezőgazdaságra és a növényvédelemre. A kémiai ökológiai alapelvek alkalmazásának szükségessége különösen sürgetővé vált az invazív fajok terjedésével, amelyek egyaránt veszélyeztetik az ökoszisztémákat és a mezőgazdasági rendszereket. A kártevők elleni védekezés során az a felismerés, hogy a rovarok miként reagálnak a növényi vegyületekre, lehetővé teszi fenntarthatóbb és célzottabb védekezési stratégiák kidolgozását, amelyek csökkenthetik a környezetet gyakran károsítjó, szélesspektrumú növényvédőszerek alkalmazását. A kémiai ökológia így egyre fontosabbá válik a modern mezőgazdaságban, nemcsak a növények védelmében, hanem az élelmiszerbiztonság előmozdításában és a biodiverzitásra gyakorolt negatív hatások csökkentésében is.

A különböző mintavételi módszerek jelentős hatással lehetnek az illékony szerves vegyületek (VOC) jellemzésére, ami befolyásolhatja az elektrofiziológiás és viselkedési kísérletek eredményeinek értelmezését is. Ezért a mintavételi eljárások optimalizálása kulcsfontosságú a pontos VOC-analízis érdekében. Tanulmányunkban négy különböző adszorbens és három mintavételi időintervallum tesztelését végeztük egy nem paraméteres statisztikai módszer, a rangkülönbségek összegzése (Sum of Ranking Differences, SRD) segítségével. Egyik fő megállapításunk az volt, hogy még egy redukált vegyületkészlet alkalmazásával is meghatározható az optimális mintavételi eljárás. Továbbá, a vegyületek kémiai csoportokba sorolásával az SRD képes rangsorolni azok a mintavételi eljárásokat, feltárva, hogy mely vegyületcsoportokat abszorbeálják legjobban az egyes módszerek.

Az elektroantennográfiás válasz amplitúdója az egyes ORN-ok receptorpotenciáljától és a válaszképes ORN-ok elektróda közeli sűrűségétől függ, különösen a gyümölcslegyekhez hasonló csápok esetében. Az elektroantennográfiás vizsgálatokhoz használt rovarcsápok preparálási módszerét az adott rovarfaj csápalakja és -struktúrája kellene, hogy meghatározza. Kísérleteink során kifejlett és nimfa *M. pruinosa* egyedekkel is sikerült elektroantennográfiás méréseket készítenünk. Az érzékelt vegyületek különböző kémiai osztályokba tartoznak, ami a *M. pruinosa* széleskörű perifériás érzékelési képességét mutatja. Érdekes módon nem találtunk a perifériás érzékenységben jelentős különbséget sem a hímek és nőstények, sem a nimfák és kifejlett egyedek között, ami arra utalhat, hogy a *M. pruinosa* az életciklusa során ugyanazokat az illékony vegyületeket használja a tájékozódás és tápnövényválasztás során. A jövőben viselkedési vizsgálatokat és terepi kísérleteket is végeznünk kell. Utóbbi vizsgálatok elengedhetetlenek a vegyületek természetes körülmények közötti teszteléséhez, mivel a természetben a többi

érzékszervre ható inger is befolyásolja a döntést. A tesztelt vegyületek skálájának bővítése mélyebb betekintést nyújthat a tápnövény illatanyag alapján történő kiválasztásának megértésébe.

A rovarcsalogatáson alapuló növényvédelem egyik fontos feltétele, hogy a csalogató anyag specifikus legyen a célfajra. Tanulmányunk megerősítette, hogy az élesztő alapú csapdák – különösen a *H. uvarum* élesztővel inokulált almalé – specifikusabbak voltak a *D. suzukii* muslicára mint az elterjedten haszált almaecetes csapdák. Ez a specifitás valószínűleg az élesztőkultúrák illékony vegyületeinek összetételéből ered. Eredményeink hangsúlyozzák az illékony anyagok összetételének fontosságát a csalétkek tervezésében, és kiemelik az élesztő-rovar kapcsolatok jelentős szerepét. A jövőben a nem célfajok dokumentációjának alapvető gyakorlatnak kellene lennie – még akkor is, ha ez idő és erőforrás igényesebb. Az ilyen adatok értékelése alapvető fontosságú a kísérletek átfogó értékelésében, különösen a biodiverzitás megőrzése miatt.

Összegzésként, e dolgozat a hagyományos kémiai ökológia munkafolyamatát követve mutat be többoldalú stratégiákat a kártevőirtás területén, különös tekintettel az invazív fajokra. A kémiai ökológia különböző szakaszaiban végzett részletes vizsgálatok – az illékony vegyületek gyűjtésétől és elemzésétől az elektrofiziológiai méréseken át a terepi csapdázásokig – célja, hogy mind azonnali, mind hosszú távú megoldásokat kínáljon a hatékony kártevőirtás érdekében.

A *Metcalfa pruinosa* elektorifiziológiás vizsgálatai rávilágítanak a kémiai ökológia szerepére a perifériás érzékelési mechanizmusok megértésében, míg a *Drosophila suzukii*-val kapcsolatos kutatás annak jelentőségét mutatják be a rovarviselkedés megfejtésében, elősegítve a célzott védekezési stratégiák kidolgozását. Ezek a mezőgazdaságra jelentős veszélyt jelentő fajok olyan specifikus kémiai ingerekre reagálnak, amelyek kihasználhatók a környezetbarát védekezési módszerekhez. Az innovatív megközelítések alkalmazásával – mint például a mintavételi eljárások optimalizálása és a szelektívebb csalétkek létrehozása – kutatásom értékes betekintést nyújt a kémiai ökológia növényvédelemben és biodiverzitás megőrzésében való alkalmazásába.

A dolgozat eredményei szélesebb körben megerősítik az értekezés bevezetőjében tett állítást: a kémiai ökológia nemcsak az alapkutatás számára fontos, hanem elengedhetetlen a modern mezőgazdaság sürgető kihívásainak kezeléséhez is. Az invazív fajok terjedésének felgyorsulásával az alkalmazkodóképes és környezetbarát kártevőirtás szükségessége egyre sürgetőbbé válik. E dolgozat eredményei hozzájárulnak ehhez a célhoz mivel gyakorlati eszközöket és stratégiákat kínálnak, amelyek integrálhatók a jövőbeni kártevőirtási gyakorlatokba, miközben megalapozzák a kémiai kölcsönhatások további kutatásának szükségességét az ökoszisztémák egyensúlyának fenntartása érdekében.

12 TABLE OF FIGURES AND TABLES

13 S1 – REFERENCES

1. Agelopoulos, N.G. and Pickett, J.A. 1998. Headspace analysis in chemical ecology: effects of different sampling methods on ratios of volatile compounds present in headspace samples. *Journal of Chemical Ecology*. **24**(7), pp.1161–1172.
2. Alma, A., Ferracini, C. and Burgio, G. 2005. Development of a sequential plan to evaluate *Neodryinus typhlocybae* (Ashmead)(Hymenoptera: Dryinidae) population associated with *Metcalfa pruinosa* (Say)(Homoptera: Flatidae) infestation in northwestern Italy. *Environmental Entomology*. **34**(4), pp.819–824.
3. Anastasaki, E., Psoma, A., Partsinevelos, G., Papachristos, D. and Milonas, P. 2021. Electrophysiological responses of *Philaenus spumarius* and *Neophilaenus campestris* females to plant volatiles. *Phytochemistry*. **189**, p.112848.
4. Arn, H., Esbjerg, P., Bues, R., Tóth, M., Szöcs, G., Guerin, P. and Rauscher, S. 1983. Field attraction of *Agrotis segetum* males in four European countries to mixtures containing three homologous acetates. *Journal of Chemical Ecology*. **9**, pp.267–276.
5. Asplen, M.K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.-L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vétek, G., Vogt, H., Walton, V.M., Yu, Y., Zappalà, L. and Desneux, N. 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*. **88**(3), pp.469–494.
6. Baker, T.C. and Cardé, R.T. 1984. Techniques for Behavioral Bioassays *In*: H. E. Hummel and T. A. Miller, eds. *Techniques in Pheromone Research* [Online]. Springer Series in Experimental Entomology. New York, NY: Springer New York, pp.45–73. [Accessed 20 June 2024]. Available from: http://link.springer.com/10.1007/978-1-4612-5220-7_2.
7. Barbosa-Cornelio, R., Cantor, F., Coy-Barrera, E. and Rodríguez, D. 2019. Tools in the Investigation of Volatile Semiochemicals on Insects: From Sampling to Statistical Analysis. *Insects*. **10**(8), p.241.
8. Bates, D., Mächler, M., Bolker, B., Walker, S., Christensen, R.H., Singmann, H. and Dai, B. 2015. *lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014*.
9. Bell, W.J. and Cardé, R.T. 2013. *Chemical ecology of insects*. Springer.
10. Bellutti, N., Gallmetzer, A., Innerebner, G., Schmidt, S., Zelger, R. and Koschier, E.H. 2018. Dietary yeast affects preference and performance in *Drosophila suzukii*. *Journal of Pest Science*. **91**(2), pp.651–660.
11. Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*. **57**(1), pp.289–300.
12. Beroza, M. and Knipling, E. 1972. Gypsy Moth Control with the Sex Attractant Pheromone: Mass trapping or permeation of the air with pheromone can prevent male gypsy moths from finding mates. *Science*. **177**(4043), pp.19–27.
13. Biasazin, T.D., Larsson Herrera, S., Kimbokota, F. and Dekker, T. 2019. Translating olfactomes into attractants: shared volatiles provide attractive bridges for polyphagy in fruit flies. *Ecology letters*. **22**(1), pp.108–118.
14. Bigiani, A., Scalera, G., Crnjar, R., Barbarossa, I.T., Magherini, P.C. and Pietra, P. 1989. Distribution and function of the antennal olfactory sensilla in *Ceratitis capitata* Wied. (Diptera, Trypetidae). *Bolletino di zoologia*. **56**(4), pp.305–311.
15. Bing, X., Gerlach, J., Loeb, G. and Buchon, N. 2018. Nutrient-Dependent Impact of Microbes on *Drosophila suzukii* Development M. J. McFall-Ngai, ed. *mBio*. **9**(2), pp.e02199-17.
16. Bueno, E., Martin, K.R., Raguso, R.A., Mcmullen, J.G., Hesler, S.P., Loeb, G.M. and Douglas, A.E. 2020. Response of Wild Spotted Wing Drosophila (*Drosophila suzukii*) to Microbial Volatiles. *Journal of Chemical Ecology*. **46**(8), pp.688–698.

17. Burkholder, W.E. and Ma, M. 1985. Pheromones for monitoring and control of stored-product insects. *Annual Review of Entomology*. **30**(1), pp.257–272.
18. Campbell, C., Pettersson, J., Pickett, J., Wadhams, L. and Woodcock, C. 1993. Spring migration of damson-hop aphid, *Phorodon humuli* (Homoptera, Aphididae), and summer host plant-derived semiochemicals released on feeding. *Journal of Chemical Ecology*. **19**, pp.1569–1576.
19. Cardé, R.T. and Elkinton, J.S. 1984. Field Trapping with Attractants: Methods and Interpretation *In*: H. E. Hummel and T. A. Miller, eds. *Techniques in Pheromone Research* [Online]. Springer Series in Experimental Entomology. New York, NY: Springer New York, pp.111–129. [Accessed 20 June 2024]. Available from: http://link.springer.com/10.1007/978-1-4612-5220-7_4.
20. Carter, M.E., Smith, M.T., Turgeon, J.J. and Harrison, R.G. 2009. Analysis of genetic diversity in an invasive population of Asian long-horned beetles in Ontario, Canada. *The Canadian Entomologist*. **141**(6), pp.582–594.
21. Castellan, I., Duménil, C., Rehermann, G., Eisenstecken, D., Bianchi, F., Robatscher, P., Spitaler, U., Favaro, R., Schmidt, S., Becher, P.G. and Angeli, S. 2024. Chemical and Electrophysiological Characterisation of Headspace Volatiles from Yeasts Attractive to *Drosophila suzukii*. *Journal of Chemical Ecology*.
22. Cha, D.H., Adams, T., Rogg, H. and Landolt, P.J. 2012. Identification and Field Evaluation of Fermentation Volatiles from Wine and Vinegar that Mediate Attraction of Spotted Wing Drosophila, *Drosophila suzukii*. *Journal of Chemical Ecology*. **38**(11), pp.1419–1431.
23. Cha, D.H., Linn Jr, C.E., Teal, P.E., Zhang, A., Roelofs, W.L. and Loeb, G.M. 2011. Eavesdropping on plant volatiles by a specialist moth: significance of ratio and concentration. *PLoS One*. **6**(2), p.e17033.
24. Chandler, J.A., Morgan Lang, J., Bhatnagar, S., Eisen, J.A. and Kopp, A. 2011. Bacterial Communities of Diverse Drosophila Species: Ecological Context of a Host–Microbe Model System H. S. Malik, ed. *PLoS Genetics*. **7**(9), p.e1002272.
25. Chen, C.-S., Zhao, C., Wu, Z.-Y., Liu, G.-F., Yu, X.-P. and Zhang, P.-J. 2021. Whitefly-induced tomato volatiles mediate host habitat location of the parasitic wasp *Encarsia formosa*, and enhance its efficacy as a bio-control agent. *Pest Management Science*. **77**(2), pp.749–757.
26. Coll Aráoz, M.V., Jacobi, V.G., Fernandez, P.C., Luft Albarracin, E., Virla, E.G., Hill, J.G. and Catalán, C.A.N. 2019. Volatiles mediate host-selection in the corn hoppers *Dalbulus maidis* (Hemiptera: Cicadellidae) and Peregrinus maidis (Hemiptera: Delphacidae). *Bulletin of Entomological Research*. **109**(05), pp.633–642.
27. Crava, C.M., Sassù, F., Tait, G., Becher, P.G. and Anfora, G. 2019. Functional transcriptome analyses of Drosophila suzukii antennae reveal mating-dependent olfaction plasticity in females. *Insect Biochemistry and Molecular Biology*. **105**, pp.51–59.
28. De Araujo, L.I., Karsten, M. and Terblanche, J.S. 2019. Exploring thermal flight responses as predictors of flight ability and geographic range size in Drosophila. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **236**, p.110532.
29. Dean, H.A. and Bailey, J.C. 1961. A Flatid Planthopper, *Metcalfa pruinosa*. *Journal of Economic Entomology*. **54**(6), pp.1104–1106.
30. Degenhardt, J., Hiltpold, I., Köllner, T.G., Frey, M., Gierl, A., Gershenzon, J., Hibbard, B.E., Ellersieck, M.R. and Turlings, T.C. 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proceedings of the National Academy of Sciences*. **106**(32), pp.13213–13218.
31. Dekker, T., Ibba, I., Siju, K.P., Stensmyr, M.C. and Hansson, B.S. 2006. Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. *Current Biology*. **16**(1), pp.101–109.
32. Derstine, N.T., Meier, L., Canlas, I., Murman, K., Cannon, S., Carrillo, D., Wallace, M. and Cooperband, M.F. 2020. Plant volatiles help mediate host plant selection and attraction of the

spotted lanternfly (Hemiptera: Fulgoridae): a generalist with a preferred host. *Environmental Entomology*. **49**(5), pp.1049–1062.
33. Deza-Durand, K.M. and Petersen, M.A. 2011. The effect of cutting direction on aroma compounds and respiration rate of fresh-cut iceberg lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology*. **61**(1), pp.83–90.
34. Dicke, M. and Baldwin, I.T. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in plant science*. **15**(3), pp.167–175.
35. Diez-Simon, C., Ammerlaan, B., Van Den Berg, M., Van Duynhoven, J., Jacobs, D., Mumm, R. and Hall, R.D. 2020. Comparison of volatile trapping techniques for the comprehensive analysis of food flavourings by Gas Chromatography-Mass Spectrometry. *Journal of Chromatography A*. **1624**, p.461191.
36. Dilawari, V. and Atwal, A. 1989. Response of mustard aphid, *Lipaphis erysimi* (Kalt.) to allylisothiocyanate.
37. Donati, I., Mauri, S., Buriani, G., Cellini, A. and Spinelli, F. 2017. Role of *Metcalfa pruinosa* as a vector for *Pseudomonas syringae* pv. *actinidiae*. *The Plant Pathology Journal*. **33**(6), p.554.
38. Dougherty, M., Guerin, P. and Ward, R. 1995. Identification of oviposition attractants for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae) in volatiles of faeces from vertebrates. *Physiological Entomology*. **20**(1), pp.23–32.
39. Dudareva, N., Negre, F., Nagegowda, D.A. and Orlova, I. 2006. Plant Volatiles: Recent Advances and Future Perspectives. *Critical Reviews in Plant Sciences*. **25**(5), pp.417–440.
40. Einzelnen, A. 1962. Elektrophysiologische untersuchungen an einzelnen geruchsrezeptoren auf den antennen des Totengrabers (Necrophorus, Coleoptera). *Z. Vgl. Physiol*. **46**, pp.212–248.
41. El-Ghany, N. 2019. Semiochemicals for controlling insect pests. *Journal of Plant Protection Research*. **59**(1).
42. El-Sayed, A., Suckling, D., Byers, J., Jang, E. and Wearing, C. 2009. Potential of "lure and kill" in long-term pest management and eradication of invasive species. *Journal of economic entomology*. **102**(3), pp.815–835.
43. Feijó Barreira, L.M., Duporté, G., Rönkkö, T., Parshintsev, J., Hartonen, K., Hyrsky, L., Heikkinen, E., Jussila, M., Kulmala, M. and Riekkola, M.-L. 2018. Field measurements of biogenic volatile organic compounds in the atmosphere using solid-phase microextraction Arrow. *Atmospheric Measurement Techniques*. **11**(2), pp.881–893.
44. Fein, B.L., Reissig, W.H. and Roelofs, W.L. 1982. Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *Journal of Chemical Ecology*. **8**(12), pp.1473–1487.
45. Gadenne, C., Groh, C., Grübel, K., Joschinski, J., Krauss, J., Krieger, J., Rössler, W. and Anton, S. 2019. Neuroanatomical correlates of mobility: sensory brain centres are bigger in winged than in wingless parthenogenetic pea aphid females. *Arthropod structure & development*. **52**, p.100883.
46. Germinara, G.S., Ganassi, S., Pistillo, M.O., Di Domenico, C., De Cristofaro, A. and Di Palma, A.M. 2017. Antennal olfactory responses of adult meadow spittlebug, *Philaenus spumarius*, to volatile organic compounds (VOCs). *PLoS One*. **12**(12), p.e0190454.
47. Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., Gavryushkin, A., Carlson, J.M., Beerenwinkel, N. and Ludington, W.B. 2018. Microbiome interactions shape host fitness. *Proceedings of the National Academy of Sciences*. **115**(51).
48. Guadagnin, M., Mori, N., Alberghini, S., Carturan, E., Girolami, V. and Bertaccini, A. 2000. Molecular evidence of phytoplasma transmission to grapevine by *Metcalfa pruinosa* (Say) in Italy *In*: *ICVG Conference*., pp.99–100.
49. Guarino, S., Arif, M.A., Millar, J.G., Colazza, S. and Peri, E. 2018. Volatile unsaturated hydrocarbons emitted by seedlings of *Brassica* species provide host location cues to *Bagrada hilaris* A. Biondi, ed. *PLOS ONE*. **13**(12), p.e0209870.

50. Guglielmino, A. and Virla, E.G. 1997. Postembryonic development and biology of *Psammotettix alienus* (Dahlbom)(Homoptera, Cicadellidae) under laboratory conditions. *Bollettino di Zoologia, Agraria e di Bachicoltura*. **29**, pp.65–80.
51. Günther, C.S. and Goddard, M.R. 2019. Do yeasts and *Drosophila* interact just by chance? *Fungal Ecology*. **38**, pp.37–43.
52. Hamby, K.A., Hernández, A., Boundy-Mills, K. and Zalom, F.G. 2012. Associations of yeasts with spotted-wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. *Applied and environmental microbiology*. **78**(14), pp.4869–4873.
53. Hansson, B. and Wicher, D. 2016. Chemical ecology in insects. *Chemosensory Transduction*., pp.29–45.
54. Hansson, B.S. and Stensmyr, M.C. 2011. Evolution of insect olfaction. *Neuron*. **72**(5), pp.698–711.
55. Hartig, F. and Hartig, M.F. 2017. Package 'dharma'. *R package*.
56. Haynes, K. and Millar, J.G. 1998. *Methods in chemical ecology. 2: Bioassay methods*. New York: Chapman & Hall.
57. Héberger, K. and Kollár-Hunek, K. 2019. Comparison of validation variants by sum of ranking differences and ANOVA. *Journal of Chemometrics*. **33**(6), p.e3104.
58. Heil, M. and Karban, R. 2010. Explaining evolution of plant communication by airborne signals. *Trends in ecology & evolution*. **25**(3), pp.137–144.
59. Hong, Y.S. and Kim, K.S. 2016. Determination of the volatile flavor components of orange and grapefruit by simultaneous distillation-extraction. *Korean Journal of Food Preservation*. **23**(1), pp.63–73.
60. Hothorn, T., Bretz, F. and Westfall, P. 2008. Simultaneous Inference in General Parametric Models. *Biometrical Journal*. **50**(3), pp.346–363.
61. Ignell, R., Couillaud, F. and Anton, S. 2001. Juvenile-hormone-mediated plasticity of aggregation behaviour and olfactory processing in adult desert locusts. *Journal of Experimental Biology*. **204**(2), pp.249–259.
62. Ioriatti, C., Bagnoli, B., Lucchi, A., Veronelli, V., and others 2004. Vine moths control by mating disruption in Italy: results and future prospects. *Redia*. **87**, pp.117–128.
63. Isaacs, R., Hardie, J., Hick, A.J., Pye, B.J., Smart, L.E., Wadhams, L.J. and Woodcock, C.M. 1993. Behavioural responses of *Aphis fabae* to isothiocyanates in the laboratory and field. *Pesticide Science*. **39**(4), pp.349–355.
64. Jacob, V.E. 2018. Current source density analysis of electroantennogram recordings: A tool for mapping the olfactory response in an insect antenna. *Frontiers in cellular neuroscience*. **12**, p.287.
65. Jiménez-Padilla, Y., Esan, E.O., Floate, K.D. and Sinclair, B.J. 2020. Persistence of diet effects on the microbiota of *Drosophila suzukii* (Diptera: Drosophilidae). *The Canadian Entomologist*. **152**(4), pp.516–531.
66. Jones, R., Fountain, M.T., Günther, C.S., Eady, P.E. and Goddard, M.R. 2021. Separate and combined *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* metabolic volatiles are attractive to *Drosophila suzukii* in the laboratory and field. *Scientific Reports*. **11**(1), p.1201.
67. Joseph, R.M. and Carlson, J.R. 2015. Drosophila Chemoreceptors: A Molecular Interface Between the Chemical World and the Brain. *Trends in Genetics*. **31**(12), pp.683–695.
68. Kaissling, K. 1986. Chemo-Electrical Transduction in Insect Olfactory Receptors. *Annual Review of Neuroscience*. **9**(1), pp.121–145.
69. Kassambara, A. 2020. Pipe-friendly framework for basic statistical tests. *R package version 0.6. 0*.
70. Keil, T.A. 1999. Morphology and development of the peripheral olfactory organs *In*: *Insect olfaction*. Springer, pp.5–47.
71. Kennedy, J.S. and Moorhouse, J.E. 1969. Laboratory observations on locust responses to wind-borne grass odour. *Entomologia Experimentalis et Applicata*. **12**(5), pp.487–503.

72. Kirkpatrick, D.M., Leach, H.L., Xu, P., Dong, K., Isaacs, R. and Gut, L.J. 2018. Comparative Antennal and Behavioral Responses of Summer and Winter Morph *Drosophila suzukii* (Diptera: Drosophilidae) to Ecologically Relevant Volatiles. *Environmental Entomology*. **47**(3), pp.700–706.
73. Klick, J., Rodriguez-Saona, C.R., Cumplido, J.H., Holdcraft, R.J., Urrutia, W.H., Da Silva, R.O., Borges, R., Mafra-Neto, A. and Seagraves, M.P. 2019. Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae) management. *Journal of Insect Science*. **19**(1), p.3.
74. Knapp, L., Mazzi, D. and Finger, R. 2021. The economic impact of *Drosophila suzukii* : perceived costs and revenue losses of Swiss cherry, plum and grape growers. *Pest Management Science*. **77**(2), pp.978–1000.
75. Knudsen, G.K., Tasin, M., Aak, A. and Thöming, G. 2018. A Wind Tunnel for Odor Mediated Insect Behavioural Assays. *Journal of Visualized Experiments*. (141), p.58385.
76. Knudsen, J.T. and Gershenzon, J. 2020. The chemical diversity of floral scent. *Biology of plant volatiles*., pp.57–78.
77. Kollár-Hunek, K. and Héberger, K. 2013. Method and model comparison by sum of ranking differences in cases of repeated observations (ties). *Chemometrics and Intelligent Laboratory Systems*. **127**, pp.139–146.
78. Landi, F., Prandini, A., Paltrinieri, S., Mori, N. and Bertaccini, A. 2007. Detection of different types of phytoplasmas in stone fruit orchards in northern Italy. *Bulletin of Insectology*. **60**(2), p.163.
79. Larsson Herrera, S., Rikk, P., Köblös, G., Szelényi, M.O., Molnár, B.P., Dekker, T. and Tasin, M. 2020. Designing a species-selective lure based on microbial volatiles to target *Lobesia botrana*. *Scientific Reports*. **10**(1), p.6512.
80. Larsson, M.C. 2016. Pheromones and other semiochemicals for monitoring rare and endangered species. *Journal of Chemical Ecology*. **42**(9), pp.853–868.
81. Lasa, R., Navarro-de-la-Fuente, L., Gschaedler-Mathis, A.C., Kirchmayr, M.R. and Williams, T. 2019. Yeast species, strains, and growth media mediate attraction of *Drosophila suzukii* (Diptera: Drosophilidae). *Insects*. **10**(8), p.228.
82. Laznik, Ž. and Trdan, S. 2018. Are synthetic volatiles, typically emitted by insect-damaged peach cultivars, navigation signals for two-spotted lady beetle (*Adalia bipunctata* L.) and green lacewing (*Chrysoperla carnea* [Stephens]) larvae? *Journal of plant diseases and protection*. **125**, pp.529–538.
83. Lee, J.C. 2010. Effect of Methyl Salicylate-Based Lures on Beneficial and Pest Arthropods in Strawberry. *Environmental Entomology*. **39**(2), pp.653–660.
84. Legaspi, J.C., Miller, N., Kanga, L., Haseeb, M. and Zanuncio, J. 2020. Attract and reward" for syrphid flies using methyl salicylate and sweet alyssum in kale in north Florida. *Subtropical Agriculture and Environments*. **71**, pp.49–52.
85. Liu, Z., Hu, T., Guo, H.-W., Liang, X.-F. and Cheng, Y.-Q. 2021. Ultrastructure of the Olfactory Sensilla across the Antennae and Maxillary Palps of *Bactrocera dorsalis* (Diptera: Tephritidae). *Insects*. **12**(4), p.289.
86. Lonchamp, J., Barry-Ryan, C. and Devereux, M. 2009. Identification of volatile quality markers of ready-to-use lettuce and cabbage. *Food Research International*. **42**(8), pp.1077–1086.
87. Matsui, K. 2006. Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology*. **9**(3), pp.274–280.
88. Mayer, C.J., Vilcinskas, A. and Gross, J. 2008. Pathogen-induced release of plant allomone manipulates vector insect behavior. *Journal of chemical ecology*. **34**, pp.1518–1522.
89. Mazzetto, F., Gonella, E., Crotti, E., Vacchini, V., Syrpas, M., Pontini, M., Mangelinckx, S., Daffonchio, D. and Alma, A. 2016. Olfactory attraction of *Drosophila suzukii* by symbiotic acetic acid bacteria. *Journal of Pest Science*. **89**, pp.783–792.

90. McCormick, A.C., Reinecke, A., Gershenzon, J. and Unsicker, S.B. 2016. Feeding experience affects the behavioral response of polyphagous gypsy moth caterpillars to herbivore-induced poplar volatiles. *Journal of chemical ecology*. **42**, pp.382–393.
91. McDonald, R.C. and Kok, L.T. 2014. A Simple Method of Detecting Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Predator Activity Using Ultraviolet-A Light. *Journal of Entomological Science*. **49**(2), pp.200–205.
92. Md Meftaul, I., Venkateswarlu, K., Dharmarajan, R., Annamalai, P. and Megharaj, M. 2020. Pesticides in the urban environment: A potential threat that knocks at the door. *Science of The Total Environment*. **711**, p.134612.
93. Mergenthaler, E., Fodor, J., Kiss, E., Bodnár, D., Kiss, B. and Viczián, O. 2020. Biological and molecular evidence for the transmission of aster yellows phytoplasma to French marigold (*Tagetes patula*) by the flatid planthopper *Metcalfa pruinosa*. *Annals of Applied Biology*. **176**(3), pp.249–256.
94. Metcalf, R.L. 1985. Plant kairomones and insect pest control. *Illinois Natural History Survey Bulletin*. **33**, pp.175–198.
95. Misztal, P.K., Lymperopoulou, D.S., Adams, R.I., Scott, R.A., Lindow, S.E., Bruns, T., Taylor, J.W., Uehling, J., Bonito, G., Vilgalys, R. and Goldstein, A.H. 2018. Emission Factors of Microbial Volatile Organic Compounds from Environmental Bacteria and Fungi. *Environmental Science & Technology*. **52**(15), pp.8272–8282.
96. Mohammed, K., Agarwal, M., Du, X.B., Newman, J. and Ren, Y. 2019. Behavioural responses of the parasitoid *Aphytis melinus* to volatiles organic compounds (VOCs) from *Aonidiella aurantii* on its host fruit Tahitian lime fruit Citrus latifolia. *Biological Control*. **133**, pp.103–109.
97. Mohammed, K., Agarwal, M., Li, B., Newman, J., Liu, T. and Ren, Y. 2020. Evaluation of d-Limonene and β-Ocimene as Attractants of *Aphytis melinus* (Hymenoptera: Aphelinidae), a Parasitoid of *Aonidiella aurantii* (Hemiptera: Diaspididae) on Citrus spp. *Insects*. **11**(1), p.44.
98. Molnár, B.P., Tóth, Z., Fejes-Tóth, A., Dekker, T. and Kárpáti, Z. 2015. Electrophysiologically-Active Maize Volatiles Attract Gravid Female European Corn Borer, *Ostrinia nubilalis*. *Journal of Chemical Ecology*. **41**(11), pp.997–1005.
99. Moon, S.-R., Cho, S.-R., Jeong, J.-W., Shin, Y.-H., Yang, J.-O., Ahn, K.-S., Yoon, C. and Kim, G.-H. 2011. Attraction response of spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) to spearmint oil. *Journal of the Korean Society for Applied Biological Chemistry*. **54**, pp.558–567.
100. Moraes, M.C., Laumann, R.A., Pareja, M., Sereno, F.T., Michereff, M.F., Birkett, M.A., Pickett, J.A. and Borges, M. 2009. Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with cis-jasmone. *Entomologia experimentalis et applicata*. **131**(2), pp.178–188.
101. Murali-Baskaran, R.K., Sharma, K.C., Kaushal, P., Kumar, J., Parthiban, P., Senthil-Nathan, S. and Mankin, R.W. 2018. Role of kairomone in biological control of crop pests-A review. *Physiological and Molecular Plant Pathology*. **101**, pp.3–15.
102. Murgier, J., Everaerts, C., Farine, J.-P. and Ferveur, J.-F. 2019. Live yeast in juvenile diet induces species-specific effects on Drosophila adult behaviour and fitness. *Scientific Reports*. **9**(1), p.8873.
103. Myrick, A.J. and Baker, T.C. 2012. Chopper-stabilized gas chromatography-electroantennography: Part I. Background, signal processing and example. *Biosensors and Bioelectronics*. **31**(1), pp.197–204.
104. Nogueira, J.M.F. 2015. Stir-bar sorptive extraction: 15 years making sample preparation more environment-friendly. *TrAC Trends in Analytical Chemistry*. **71**, pp.214–223.
105. Nomaani, R.S.S.A., Hossain, M.A., Weli, A.M., Al-Riyami, Q. and Al-Sabahi, J.N. 2013. Chemical composition of essential oils and in vitro antioxidant activity of fresh and dry leaves crude extracts of medicinal plant of *Lactuca Sativa* L. native to Sultanate of Oman. *Asian Pacific Journal of Tropical Biomedicine*. **3**(5), pp.353–357.

106. Nottingham, S.F. and Hardie, J. 1993. Flight behaviour of the black bean aphid, *Aphis fabae*, and the cabbage aphid, Brevicoryne brassicae, in host and non-host plant odour. *Physiological Entomology*. **18**(4), pp.389–394.
107. Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. 1991. Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of chemical ecology*. **17**, pp.1231–1242.
108. Ochiai, N., Tsunokawa, J., Sasamoto, K. and Hoffmann, A. 2014. Multi-volatile method for aroma analysis using sequential dynamic headspace sampling with an application to brewed coffee. *Journal of Chromatography A*. **1371**, pp.65–73.
109. Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., and others 2019. vegan: Community Ecology Package. R package version 2.5-6.
110. Palanca, L., Gaskett, A.C., Günther, C.S., Newcomb, R.D. and Goddard, M.R. 2013. Quantifying Variation in the Ability of Yeasts to Attract *Drosophila melanogaster* F. Leulier, ed. *PLoS ONE*. **8**(9), p.e75332.
111. Pare, P.W. and Tumlinson, J.H. 1997. De Novo Biosynthesis of Volatiles Induced by Insect Herbivory in Cotton Plants. *Plant Physiology*. **114**(4), pp.1161–1167.
112. Piñero, J.C., Barrett, B.A., Bolton, L.G. and Follett, P.A. 2019. β-cyclocitral synergizes the response of adult *Drosophila suzukii* (Diptera: Drosophilidae) to fruit juices and isoamyl acetate in a sex-dependent manner. *Scientific Reports*. **9**(1), p.10574.
113. Polizzi, V., Adams, A., Picco, A.M., Adriaens, E., Lenoir, J., Van Peteghem, C., De Saeger, S. and De Kimpe, N. 2011. Influence of environmental conditions on production of volatiles by Trichoderma atroviride in relation with the sick building syndrome. *Building and Environment*. **46**(4), pp.945–954.
114. Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J. and Turlings, T.C. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*. **434**(7034), pp.732–737.
115. Rehermann, G., Spitaler, U., Sahle, K., Cossu, C.S., Donne, L.D., Bianchi, F., Eisenstecken, D., Angeli, S., Schmidt, S. and Becher, P.G. 2022. Behavioral manipulation of *Drosophila suzukii* for pest control: high attraction to yeast enhances insecticide efficacy when applied on leaves. *Pest Management Science*. **78**(3), pp.896–904.
116. Revadi, S., Eccher, F., Mazzoni, V., Al-Ani, S., Carlin, S., Vrhovsek, U., Anfora, G., and others 2013. Olfactory responses of *Drosophila suzukii* to host plant volatiles. *Olfactory responses of Drosophila suzukii to host plant volatiles.* **91**, pp.309–313.
117. Riolo, P., Minuz, R.L., Anfora, G., Stacconi, M.V.R., Carlin, S., Isidoro, N. and Romani, R. 2012. Perception of host plant volatiles in *Hyalesthes obsoletus*: behavior, morphology, and electrophysiology. *Journal of Chemical Ecology*. **38**, pp.1017–1030.
118. Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D. and Ripley, M.B. 2013. Package 'mass'. *Cran r*. **538**, pp.113–120.
119. Robert, C.A.M., Erb, M., Hiltpold, I., Hibbard, B.E., Gaillard, M.D.P., Bilat, J., Degenhardt, J., Cambet-Petit-Jean, X., Turlings, T.C.J. and Zwahlen, C. 2013. Genetically engineered maize plants reveal distinct costs and benefits of constitutive volatile emissions in the field. *Plant Biotechnology Journal*. **11**(5), pp.628–639.
120. Roberts, J.M., Clunie, B.J., Leather, S.R., Harris, W.E. and Pope, T.W. 2023. Scents and sensibility: Best practice in insect olfactometer bioassays. *Entomologia Experimentalis et Applicata*. **171**(11), pp.808–820.
121. Romani, R., Stacconi, M.V.R., Riolo, P. and Isidoro, N. 2009. The sensory structures of the antennal flagellum in *Hyalesthes obsoletus* (Hemiptera: Fulgoromorpha: Cixiidae): a functional reduction? *Arthropod Structure & Development*. **38**(6), pp.473–483.
122. Sato, K. and Touhara*, K. 2009. Insect olfaction: receptors, signal transduction, and behavior. *Chemosensory systems in mammals, fishes, and insects*., pp.203–220.

123. Schauff, M.E. 2001. Collecting and preserving insects and mites: techniques and tools. Systematic Entomology Laboratory, USDA.
124. Schneider, D. 1957. Elektrophysiologische Untersuchungen von Chemo- und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori* L. *Zeitschrift f*[illegible]*r Vergleichende Physiologie*. **40**(1), pp.8–41.
125. Schneider, D. 1964. Insect Antennae. *Annual Review of Entomology*. **9**(1), pp.103–122.
126. Schneider, D. and Boeckh, J. 1962. Rezeptorpotential und Nervenimpulse einzelner olfaktorischer Sensillen der Insektenantenne. *Zeitschrift f*[illegible]*r Vergleichende Physiologie*. **45**(4), pp.405–412.
127. Schneider, D. and Hecker, E. 1956. Zur Elektrophysiologie der Antenne des Seidenspinners *Bombyx mori* bei Reizung mit angereicherten Extrakten des Sexuallockstoffes. *Zeitschrift für Naturforschung B*. **11**(3), pp.121–124.
128. Schuman, M.C., Barthel, K. and Baldwin, I.T. 2012. Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *eLife*. **1**, p.e00007.
129. Seo, H., Park, D., Hwang, I. and Choi, Y. 2019. Host plants of *Metcalfa pruinosa* (Say)(Hemiptera: Flatidae) nymph and adult. *Korean Journal of applied entomology*. **58**(4), pp.363–380.
130. Sharma, K.R., Enzmann, B.L., Schmidt, Y., Moore, D., Jones, G.R., Parker, J., Berger, S.L., Reinberg, D., Zwiebel, L.J., Breit, B., and others 2015. Cuticular hydrocarbon pheromones for social behavior and their coding in the ant antenna. *Cell reports*. **12**(8), pp.1261–1271.
131. Shenoy, A.R. 2021. Grafify: an R package for easy graphs, ANOVAs and post-hoc comparisons. Zenodo Geneva, Switzerland:
132. Shuttleworth, A. and Johnson, S.D. 2022. GC-MS/FID/EAD: A method for combining mass spectrometry with gas chromatography-electroantennographic detection. *Frontiers in Ecology and Evolution*. **10**, p.1042732.
133. Silva, R. and Clarke, A.R. 2020. The "sequential cues hypothesis": A conceptual model to explain host location and ranking by polyphagous herbivores. *Insect science*. **27**(6), pp.1136–1147.
134. Spaethe, J., Brockmann, A., Halbig, C. and Tautz, J. 2007. Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften*. **94**(9), pp.733–739.
135. Spitaler, U., Bianchi, F., Eisenstecken, D., Castellan, I., Angeli, S., Dordevic, N., Robatscher, P., Vogel, R.F., Koschier, E.H. and Schmidt, S. 2020. Yeast species affects feeding and fitness of *Drosophila suzukii* adults. *Journal of Pest Science*. **93**, pp.1295–1309.
136. Stensmyr, M.C., Dweck, H.K.M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S., Wicher, D., Sachse, S., Knaden, M., Becher, P.G., Seki, Y. and Hansson, B.S. 2012. A Conserved Dedicated Olfactory Circuit for Detecting Harmful Microbes in Drosophila. *Cell*. **151**(6), pp.1345–1357.
137. Świerczewski, D., Woźnica, A.J., Smulski, T. and Stroiński, A. 2022. First report of the Nearctic planthopper *Metcalfa pruinosa* (Say, 1830) in Poland, its current status and potential threats (Hemiptera: Fulgoromorpha: Flatidae). *Journal of Plant Protection Research*.
138. Swoboda-Bhattarai, K.A., McPhie, D.R. and Burrack, H.J. 2017. Reproductive Status of *Drosophila suzukii* (Diptera: Drosophilidae) Females Influences Attraction to Fermentation-Based Baits and Ripe Fruits. *Journal of Economic Entomology*. **110**(4), pp.1648–1652.
139. Szanyi, S., Molnár, A., Szanyi, K., Tóth, M., Jósvai, J.K., Varga, Z. and Nagy, A. 2024. Semiochemical-baited traps as a new method supplementing light traps for faunistic and ecological studies of Macroheterocera (Lepidoptera). *Scientific Reports*. **14**(1), p.20212.
140. Tasin, M., Cappellin, L. and Biasioli, F. 2012. Fast Direct Injection Mass-Spectrometric Characterization of Stimuli for Insect Electrophysiology by Proton Transfer Reaction-Time of Flight Mass-Spectrometry (PTR-ToF-MS). *Sensors*. **12**(4), pp.4091–4104.

141. Tholl, D., Hossain, O., Weinhold, A., Röse, U.S.R. and Wei, Q. 2021. Trends and applications in plant volatile sampling and analysis. *The Plant Journal*. **106**(2), pp.314–325.
142. Turlings, T.C., Tumlinson, J.H. and Lewis, W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*. **250**(4985), pp.1251–1253.
143. Turlings, T.C.J. and Erb, M. 2018. Tritrophic Interactions Mediated by Herbivore-Induced Plant Volatiles: Mechanisms, Ecological Relevance, and Application Potential. *Annual Review of Entomology*. **63**(1), pp.433–452.
144. Ukeh, D.A., Birkett, M.A., Bruce, T.J., Allan, E.J., Pickett, J.A. and Mordue, A.J. 2010. Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. *Pest Management Science: formerly Pesticide Science*. **66**(1), pp.44–50.
145. Vuts, J., Koczor, S., Imrei, Z., Jósvai, J.K., Lohonyai, Z., Molnár, B.P., Kárpáti, Z., Sz\Hocs, G. and Tóth, M. 2018. Módszerek a kémiai ökológiában. *Növényvédelem*. **54**(3), pp.89–109.
146. Wallingford, A.K., Cha, D.H. and Loeb, G.M. 2018. Evaluating a push–pull strategy for management of *Drosophila suzukii* Matsumura in red raspberry. *Pest Management Science*. **74**(1), pp.120–125.
147. Wallingford, A.K., Connelly, H.L., Dore Brind'Amour, G., Boucher, M.T., Mafra-Neto, A. and Loeb, G.M. 2016. Field evaluation of an oviposition deterrent for management of spotted-wing drosophila, *Drosophila suzukii*, and potential nontarget effects. *Journal of economic entomology*. **109**(4), pp.1779–1784.
148. Wang, R.-R., Liu, J.-J., Li, X.-Y., Liang, A.-P. and Bourgoin, T. 2018. Relating antennal sensilla diversity and possible species behaviour in the planthopper pest *Lycorma delicatula* (Hemiptera: Fulgoromorpha: Fulgoridae). *PLoS One*. **13**(3), p.e0194995.
149. Weintraub, P.G. and Beanland, L. 2006. Insect vectors of phytoplasmas. *Annual Review of Entomology*. **51**(1), pp.91–111.
150. Wickham, H. and Wickham, H. 2016. *Data analysis*. Springer.
151. Witzgall, P., Kirsch, P. and Cork, A. 2010. Sex Pheromones and Their Impact on Pest Management. *Journal of Chemical Ecology*. **36**(1), pp.80–100.
152. Wong, J.S., Wallingford, A.K., Loeb, G.M. and Lee, J.C. 2018. Physiological status of *Drosophila suzukii* (Diptera: Drosophilidae) affects their response to attractive odours. *Journal of Applied Entomology*. **142**(5), pp.473–482.
153. Yoon, C., Moon, S.-R., Jeong, J.-W., Shin, Y.-H., Cho, S.-R., Ahn, K.-S., Yang, J.-O. and Kim, G.-H. 2011. Repellency of lavender oil and linalool against spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) and their electrophysiological responses. *Journal of Asia-Pacific Entomology*. **14**(4), pp.411–416.
154. Youn, Y.N. 2002. Electroantennogram Responses of *Nilaparvata lugens* (Homoptera: Delphacidae) to Plant Volatile Compounds. *Journal of Economic Entomology*. **95**(2), pp.269–277.
155. Zhang, Q. and Schlyter, F. 2004. Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. *Agricultural and Forest Entomology*. **6**(1), pp.1–20.
156. Zhang, Z. and Chen, Z. 2015. Non-host plant essential oil volatiles with potential for a 'push-pull' strategy to control the tea green leafhopper, *Empoasca vitis*. *Entomologia Experimentalis et Applicata*. **156**(1), pp.77–87.
157. Zhu, Q., Wu, N., Brożek, J. and Dai, W. 2019. Antennal morphology and sexual dimorphism of antennal sensilla in *Callitettix versicolor* (Fabricius)(Hemiptera: Cercopidae). *Insects*. **10**(2), p.56.

14 TABLE S2

Table S2 *Identified volatile organic compounds in* Lactuca sativa *were cataloged with their retention indices. Italicized retention indices indicate those derived from the NIST webbook, and the 20 most intensive compounds are highlighted in bold. "nd" signifies that no data is available for certain entries.*

RT (min)	RI (calculated)	Compounds name	Formula	CAS number	area (%)	Match factor (%)
2.99	*720*	2,2-dimethylhexane	C_8H_{18}	590-73-8	0.18	>90%
3.04	*721*	methylcyclohexane	C_7H_{14}	108-87-2	0.41	>90%
3.08	*735*	2,5-dimethylhexane	C_8H_{18}	592-13-2	0.20	>95%
3.11	*731*	2,4-dimethylhexane	C_8H_{18}	589-43-5	0.40	>95%
3.15	*733*	ethylcyclopentane	C_7H_{14}	1640-89-7	0.09	>95%
3.21	*749*	2,3,3-trimethylpentane	C_8H_{18}	560-21-4	0.14	>95%
3.43	*774*	2,3-dimethylhexane	C_8H_{18}	584-94-1	0.12	>95%
3.49	*778*	2-methylheptane	C_8H_{18}	592-27-8	0.26	>95%
3.56	*776*	toluene	C7H8	108-88-3	0.15	>90%
3.60	*776*	3-methylheptane	C_8H_{18}	589-81-1	0.30	>90%
3.77	*775*	3-hexanone	$C_6H_{12}O$	589-38-8	0.04	>90%
3.83	*791*	2-hexanone	$C_6H_{12}O$	591-78-6	0.06	>85%
3.91	*797*	3-hexanol	$C_6H_{14}O$	623-37-0	0.04	>85%
3.99	803	octane	C_8H_{18}	111-65-9	0.15	~90%
4.12	810	1,3(*trans*)-dimethylcyclohexane	C_8H_{16}	2207-03-6	0.03	>85%
4.64	836	2,4-dimethylheptane	C_9H_{20}	2213-23-2	0.02	~85%
4.62	835	ethylcyclohexane	C_8H_{16}	1678-91-7	0.09	>90%
4.68	838	cyclogeraniolane	C_9H_{18}	3073-66-3	0.03	>85%
4.73	841	2,4-dimethyl-1-heptene	C_9H_{18}	19549-87-2	0.07	~90%
5.00	854	1,2,4-trimethylcyclohexane	C_9H_{18}	2234-75-5	0.08	>90%
5.16	863	ethylbenzene	C_8H_{10}	100-41-4	0.28	>90%
5.32	871	*o*-xylene	C_8H_{10}	95-47-6	0.56	>95%
5.59	885	1,1,2-trimethylcyclohexane	C_9H_{18}	7094-26-0	0.22	>95%
5.71	891	1-ethyl-4-methylcyclohexane	C_9H_{18}	3728-56-1	0.15	>95%
5.78	894	styrene	C_8H_8	100-42-5	0.17	>90%

5.84	897	*p*-xylene	C_8H_{10}	108-38-3	0.21	>95%
5.95	902	nonane	C_9H_{20}	111-84-2	0.25	>95%
6.07	907	1,2,3-trimethylcyclohexane	C_9H_{18}	1678-97-3	0.08	>95%
6.14	910	1-ethyl-3-methylcyclohexane	C_9H_{18}	3728-55-0	0.25	>95%
6.22	913	1-methyl-cis-4-ethylcyclohexane	C_9H_{18}	4926-78-7	0.08	>90%
6.45	922	1,3-dimethyl-2-(1-methylethylidene)cyclopentane	$C_{10}H_{18}$	61142-31-2	0.12	>80%
6.69	932	propylcyclohexane	C_9H_{18}	1678-92-8	0.32	>90%
6.76	934	2,6-dimethyloctane	$C_{10}H_{22}$	3051-30-1	0.26	~95%
6.81	936	*α*-pinene	$C_{10}H_{16}$	80-56-8	0.64	>95%
6.95	942	3-ethyl-2-methylheptane	$C_{10}H_{22}$	14676-29-0	0.54	>80%
7.18	951	*β*-pinene	$C_{10}H_{16}$	127-91-3	0.03	~75%
7.30	956	isocumene	C_9H_{12}	103-65-1	0.30	~80%
7.50	963	*p*-ethyltoluene	C_9H_{12}	622-96-8	0.25	>95%
7.53	965	2-methylnonane	$C_{10}H_{22}$	871-83-0	0.69	>90%
7.68	970	mesitylene	C_9H_{12}	108-67-8	0.38	~95%
7.71	972	3-methylnonane	$C_{10}H_{22}$	5911-04-6	0.42	>95%
7.79	975	*trans*-*p*-menthane	$C_{10}H_{20}$	1678-82-6	0.29	>90%
7.98	982	*o*-ethyltoluene	C_9H_{12}	611-14-3	0.10	>90%
8.13	988	*cis*-*p*-menthane	$C_{10}H_{20}$	6069-98-3	0.37	>85%
8.21	992	*cis*-octahydro-1h-indene	C_9H_{16}	4551-51-3	0.97	>95%
8.34	**997**	**benzene, 1,2,3-trimethyl-**	**C_9H_{12}**	**526-73-8**	**1.39**	**>95%**
8.48	**1002**	**decane**	**$C_{10}H_{22}$**	**124-18-5**	**3.47**	**>95%**
8.94	1019	(±) menthol	$C_{10}H_{20}O$	15356-70-4	0.39	>85%
9.12	**1025**	**4-methyldecane**	**$C_{11}H_{24}$**	**2847-72-5**	**2.04**	**>85%**
9.19	1027	2-cyclohexylbutane	$C_{10}H_{20}$	7058-01-7	0.36	>85%
9.31	1032	D-limonene	$C_{10}H_{16}$	5989-27-5	0.75	>95%
9.39	1034	butylcyclohexane	$C_{10}H_{20}$	1678-93-9	0.61	>90%
9.77	**1048**	**1,2-dimethylcyclooctene**	**$C_{10}H_{18}$**	**54299-96-6**	**0.86**	**>85%**
9.85	1051	cis-*β*-ocimene	$C_{10}H_{16}$	3338-55-4	0.12	>95%
10.04	1058	naphthan	$C_{10}H_{18}$	91-17-8	0.63	>90%
10.09	1060	2,5-dimethylnonane	$C_{11}H_{24}$	17302-27-1	0.83	>80%
10.23	**1064**	**4,7-methanoindan, hexahydro-**	**$C_{10}H_{16}$**	**6004-38-2**	**3.48**	**>95%**

10.75	**1083**	**1,1'-bicyclopentyl**	**$C_{10}H_{18}$**	**1636-39-1**	**1.76**	**>95%**
11.07	**1095**	**3-tert-butyltoluene**	**$C_{11}H_{16}$**	**1075-38-3**	**0.53**	**>80%**
11.14	**1097**	**unknown1 (135 *m/z*)**			**1.12**	
11.30	1103	undecane	$C_{11}H_{24}$	1120-21-4	3.22	**>90%**
11.41	1107	*cis*-3-methyl-endo-tricyclo[5.2.1.0(2.6)]decane	$C_{11}H_{18}$	1000215-29-0 999067-42-8	0.10	>85%
11.58	1113	*cis*-4-methyl-exo-tricyclo[5.2.1.0(2.6)]decane	$C_{11}H_{18}$	999067-42-9	0.49	>85%
12.29	1138	2-methyldecalin	$C_{11}H_{20}$	2958-76-1	0.72	>90%
12.44	1143	6,6-dimethyl-cyclooct-4-enone	$C_{10}H_{16}O$	999070-93-3	0.69	>90%
12.75	1154	tricyclo[5.2.1.0(2,6)]decane, 4-methyl-	$C_{11}H_{18}$	2000073-34-9	0.70	>90%
13.39	**1176**	**toluene, p-(1-ethylpropyl)-**	**$C_{12}H_{18}$**	**22975-58-2**	**0.32**	**>85%**
13.65	1186	benzene, 1-methyl-2-(1-ethylpropyl)-	$C_{12}H_{18}$	54410-74.1	1.03	>85%
14.15	**1203**	**benzene, 1,4-dimethyl-2-(2-methylpropyl)-**	**$C_{12}H_{18}$**	**55669-88-0**	**1.84**	**>85%**
17.51	**1326**	**2,7-dimethyltetralin**	**$C_{12}H_{16}$**	**13065-07-1**	**1.73**	**>85%**
17.66	**1332**	**6-ethyltetralin**	**$C_{12}H_{16}$**	**22531-20-0**	**2.10**	**>85%**
17.87	**1339**	**5-ethyltetralin**	**$C_{12}H_{16}$**	**42775-75-7**	**1.64**	**>85%**
20.09	1426	*β*-caryophyllen	$C_{15}H_{24}$	87-44-5	0.25	~90%
20.32	1435	(-)-isolongifolol, methyl ether	$C_{16}H_{28}O$	999281-62-4	0.24	>85%
20.52	1443	corymbolone	$C_{15}H_{24}O_2$	97094.19-4	0.16	>85%
21.14	1467	*α*-humulene	$C_{15}H_{24}$	6753-98-6	0.26	~80%
21.71	1488	*γ*-elemene	$C_{15}H_{24}$	3242-08-8	0.44	>75%
22.50	**1525**	***β*-humulene**	**$C_{15}H_{24}$**	**116-04-1**	**1.26**	**>80%**
23.55	**1568**	**unknown2 (135 *m/z)***	**-**	**-**	**4.43**	**-**
23.68	**1573**	**longifolene-i2**	**$C_{15}H_{24}$**	**1000162-76-7**	**4.88**	**~90%**
24.38	**1606**	**7-octylidenebicyclo[4.1.0]heptane**	**$C_{15}H_{26}$**	**82253-11-0**	**1.42**	**>85%**
25.08	**1636**	**1,4-methanobenzocyclodecene, 1,2,3,4,4a,5,8,9,12,12a-decahydro-**	**$C_{15}H_{22}$**	**74708-73-9**	**8.23**	**~90%**
25.30	**1645**	**cyclobuta[1,2:3,4]dicyclooctene, 1,2,5,6,6a,6b,7,8,11,12,12a,12b-dodecahydro-, (6a.alpha.,6b.alpha.,12a.beta.,12b.beta.)-**	**$C_{16}H_{24}$**	**61233-68-9**	**1.48**	**>85%**

15 TABLE S3

Table S3 *Chemical composition of* Tagetes patula, Ailanthus altissima *and* Aristolochia clematis*: the compound's calculated (Kováts index) and published retention indices, relative areas of MS, and their concentrations based on FID data (ng/µl).*

Compound	Calculated RI	NIST RI	MS area %			FID ng/µl		
			Tagetes patula	*Ailanthus altissima*	*Aristolochia clematis*	*Tagetes patula*	*Ailanthus altissima*	*Aristolochia clematis*
alkane								
4-methyloctane	-	864	0.139	-	-			
aromatic								
p-xylene	-	889	0.161	0.190	0.022			
benzaldehyde	966	964	0.545	0.331	4.023			
benzyl alcohol	1053	1036	-	0.156	-			
carboxylic acid								
hexanoic acid	979	961	-	0.265	-			
diterpene								
neophytadiene	1841	1837	1.982	-	-			
ester								
(*Z*)-3-hexenyl acetate*	1008	1001	0.019	7.740	7.314	11.6	3.7	124.6
2-hexenyl acetate	1029	1017	-	0.186	-			
methyl benzoate	1124	1093	-	0.273	-			
methyl salicylate*	1202	1192	2.344	10.129	-	9.3	17.5	0
bornyl acetate	1296	1287	-	-	4.311	0	0	60.5
(*Z*)-3-hexenyl tiglate	1352	1322	-	0.214	-			
myrtenyl acetate	1339	1335	-	-	0.488			
(*Z*)-3-hexenyl benzoate	1594	1615	-	0.542	-			
methyl jasmonate	1662	1644	0.201	-	-			
o-methylanisole	1017	1007	-	-	0.190			
o-dimethoxybenzene	1150	1146	-	-	0.132			
fatty alcohol								
2-pentenol	-	774	-	0.551	-			
2-hexenol	-	856	-	1.242	-			
(*E*)-3-hexenol	-	857	-	0.720	0.036	0	21.3	3.8
(*Z*)-3-hexenol*	-	865	2.719	61.781	7.936	24.8	785.2	519.7
1-hexanol*	-	867	0.330	1.696	0.210	5.8	21.7	21.5
2-heptanol	902	890	0.093	-	-			
2-ethylhexanol	1030	1025	0.507	0.371	-	23.2	0.5	0
fatty aldehyde	-	801	-	0.812	-	0	21.5	0

Compound	Calculated RI	NIST RI	MS area %			FID ng/µl		
			Tagetes patula	*Ailanthus altissima*	*Aristolochia clematis*	*Tagetes patula*	*Ailanthus altissima*	*Aristolochia clematis*
hexanal								
2-hexenal	-	854	0.049	-	-	15.1	0	0
octanal	1016	1003	-	0.085	-	0	9.9	0
nonanal	1130	1102	-	0.118	-	0	6.0	0
decanal	1234	1207	-	0.123	-			
ketone								
3-octanone	991	982	-	-	0.036			
sulcatone	989	991	0.130	-	-			
(*Z*)-jasmone	1407	1400	0.254	0.390	-			
lactone								
lavender lactone	1048	1041	-	-	0.107	0	0	156.4
monoterpene								
α-pinene	941	937	-	0.086	1.063			
camphene	957	953	-	0.243	1.658	0	0.7	3.7
sabinene	980	972	0.085	0.119	0.470			
β-pinene	985	981	-	0.133	0.846			
β-myrcene*	1000	992	0.035	-	1.039	20.5	0	15.5
α-phellandrene	1010	1003	1.626	-	0.037			
limonene*	1035	1027	2.474	-	1.288			
(*Z*)-*β*-ocimene*	1040	1037	10.271	-	9.881	90.2	0	39.5
(E)-*β*-ocimene*	1051	1054	1.152	0.350	1.626	9.8	16.6	17.0
DMNT*	1118	1110	2.685	2.065	-	2.7	11.9	0
α-terpinolene	1094	1092	5.359	-	-			
terpene_1	1083	-	2.456	-	2.393	1.8	0	63.6
terpene_2	1125	-	0.304	-	0.491			
cosmene	1135	1132	2.440	-	3.609	15.8	0	0
allo-ocimene	1132	1132	0.444	-	2.363			
camphor	1158	1145	-	-	4.448	0	0	80.7
monoterpenoid								
eucalyptol*	1038	1032	2.728	-	1.544	1.2	0	7.6
linalool*	1102	1101	0.707	0.159	1.446	6.7	0.4	23.7
myroxide	1144	1138	4.207	-	-	55.6	0	0
p-mentha-1,5-dien-8-ol	1173	1170	0.565	-	1.816	4.1	0	12.8
(*E*)-p-mentha-1(7),8-dien-2-ol	1129	1185	0.138	-	-			
p-cymen-8-ol	1191	1189	0.618	-	-			

Compound	Calculated RI	NIST RI	MS area %			FID ng/µl		
			Tagetes patula	*Ailanthus altissima*	*Aristolochia clematis*	*Tagetes patula*	*Ailanthus altissima*	*Aristolochia clematis*
α-terpineol	1197	1190	-	-	0.345			
(*E*,*E*)-2,6-dimethyl-3,5,7-octatriene-2-ol	1202	1209	3.717	-	3.860			
trans-2-caren-4-ol	1210	1222	3.566	0.099	4.014			
piperitone*	1263	1251	3.530	-	-	117.2	0	0
piperitenone	1353	1340	0.386	-	-			
ethyllinalool	1198	1181	0.191	-	-			
dendrolasin	1584	1581	1.787	-	-	12.5	0	0
N-heterocyclic compound								
indole	1329	1300	-	0.095	-			
sesquiterpene								
α-cubebene	1361	1348	-	-	1.068			
α-copaene*	1390	1375	0.209	-	1.029	1.9	0	7.9
β-isocomene	1427	1384	0.245	-	-			
β-elemene	1403	1392	1.409	-	3.432			
α-cedrene	1431	1398	0.178	-	-			
β-caryophyllene*	1438	1418	26.678	1.417	9.209	504.3	4.7	151.3
α-humulene	1471	1452	0.487	0.275	1.178			
β-farnesene	1461	1459	4.820	-	-			
α-curcumene	1491	1484	0.173	-	-			
germacrene D	1498	1485	4.062	2.507	14.376	44.7	7.6	328.1
α-farnesene	1530	1507	-	3.904	-			
β-bisabolene	1517	1511	0.267	-	-			
β-cadinene	1509	1515	-	0.091	-			
δ-cadinene	1531	1521	-	-	0.298			
γ-cadinene	1538	1524	-	-	0.369			
sesquiterpenoid								
caryophyllene oxide	1605	1589	0.314	-	-			

*Identified with synthetic standards.

16 ACKNOWLEDGMENTS

Completing this PhD journey has been a challenging but incredibly rewarding experience, and it would not have been possible without the support and encouragement of many people.

First and foremost, I would like to express my deepest gratitude to my supervisor, Béla Péter Molnár and Gábor Vétek for their unwavering support, invaluable guidance, and encouragement throughout the course of my research. Their expertise, patience, and insightful feedback have been instrumental in shaping both this thesis and my growth as a researcher.

Special thanks go to my colleagues and fellow researchers Anna, Gergő, Sanyi, Feri, Dalma at HUN-REN Centre for Agricultural Research Plant Protection Institute, whose camaraderie and collaboration made this journey so much more fun. I would also like to thank the partners who contributed to the data collection and analysis phases of my work.

I am deeply appreciative of the multiple financial support provided by New National Excellence Program of the Ministry for Culture and Innovation from The Source of the National Research, Development and Innovation Fund and to the finantial support of GINOP-2.3.2-15-2016-00051 E-nose research group - analysis of natural odor patterns and utilization in agricultural sciences which made my research possible.

On a personal note, I am forever grateful to my mom for her unconditional support, understanding, and love during the most challenging moments of this journey. Her belief in me has been a constant source of motivation. Special thanks to Zugligeti Bölcsőde and the dedicated caregivers – Reni, Melitta, Marica, Enikő, Rita – who took care of my sons with love and professionalism, allowing me to focus on my research with peace of mind. Your support has been invaluable to our family, and I will always be grateful for your care.

Lastly, I would like to acknowledge the countless individuals whose research and discoveries laid the foundation for my own work. It is through the collective efforts of the scientific community that we continue to advance our understanding of the world.

Thank you all.