Mapping volatiles that induce sensory responses in *Delia antiqua*: comparative identification across a set of crops

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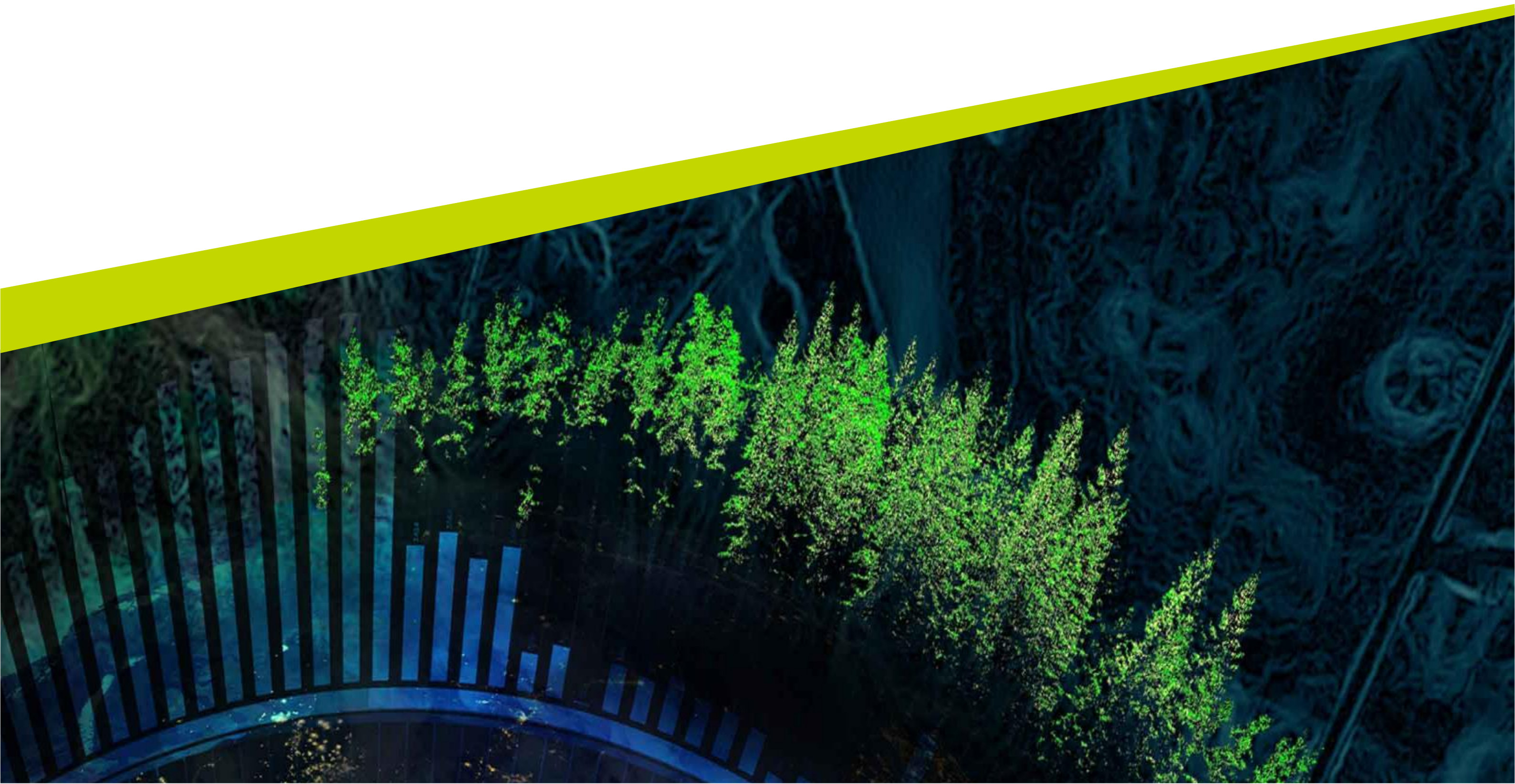

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Acknowledgements

Those who know me know that I will not have a lot of things to say under this section of my thesis. However this year has not been like all others personally and I would like to thank all of those who have made it possible for me to go on and support me in dire times of need.

Thank you of course my dear supervisors Sebastian and Teun who have spent endless hours with me trying to get this thesis to work, rearing insects, fiddling with failing lab equipment, fixing broken code and lost shipments, thank you so much. Colleagues who have welcomed me to the department with knowledge, chit chat and smiles made it worth coming to the department even on bad days.

And to others who have helped me in the moments of darkness and hopelessness this year, you know who you are.

Till mamma och pappa, tack, jag älskar er båda.

Abstract

The urge to combat climate change and the rapid degradation of agroecosystems puts high pressure on the development of novel tools to make an applied and theoretical change in the world. There is a vision on sustainability through Agenda 2030 which aligns with FAOs: the 10 elements of agroecology. Higher degrees of cooperation and collaboration for data-sharing, open access and open information could prove pivotal to solving some of said problems. Pest management is one source of the degradation of biodiversity and decline in health of both workers and the agroecosystems. Novel tools for the management of pests are necessary to reduce the impact of these degradations as the current practices and methods are unsustainable. Chemical ecology is a field of study which means to explore alternatives to the current pest management practices and the development of novel tools. Through the use of high-throughput systems it is possible to map volatilomes and identify volatile organic compounds which attract certain insect pests and make species specific lures to be utilised in novel tools for management of insect pests. One such pest is *Delia antiqua*, the onion fly. Through GC-MS and GC-EAD a set of certain sulphuric compounds in yellow onion could be identified that elicited strong electrophysiological responses in *D. antiqua*. Meanwhile, did *Drosophila melanogaster* show no response to sulphuric compounds, indicating niche-driven or phylogenetic olfactory divergence between the species. Further, the analyses of olfactory responses utilised existing open- and closed-access databases and R packages, which underlines the importance of collaboration in developing and utilising such tools to accelerate the read from research to impact. To develop species specific lure-traps more comparative research is needed across various insect species as well as host crops and it has to be done in a transparent and cooperative nature.

Keywords: attractants, gc-ead, gc-ms, olfactomics, volatiles, databases, drosophila, delia

Foreword

During the time I have spent in the agroecology master's I have taken my prior knowledge on food science, biology, chemistry, sustainability and cooperation in a new direction. When I finished my bachelor's in food science I was certain that I wanted to know how food was grown on a deeper level and how to do it in a sustainable way. Low and behold I had found the master's in agroecology at campus Alnarp. It was a chance for me to gain knowledge on the topics I held dear and learn strategies on how I could develop my ideas into something concrete. I especially like the focus on holistic systems thinking since I believe that interaction and cooperation for increased sustainability in all levels of society is utmost important. When I was presented with a project from my supervisors last autumn in 2021 for the practical research training course I felt like I had hit a jackpot. A project where I could research chemistry and biology in a setting of creating more sustainable agricultural practices for farmers as well as interact with them in person, I almost could not believe it. My time during that course as well as during the work for my thesis has taught me much on chemistry, evolution, ecology, cooperative work, programming, working in labs, rearing plants and insects, socialising with other researchers and so much more. At the same time I have taken an agroecological perspective on it, talking with farmers who are struggling with pests, interacting with hushållningssällskapet (national body which acts as hub for the development of rural Sweden) to gain knowledge that farmers report to have problems with and lastly to share the knowledge and data that we have generated to our colleagues through meetings, daily communication and visits. It has been an amazing time and I feel like I am ready to move onto the next step which I have been obsessed with since I was a child, to become a doctor of science through a PhD program continuing my search for knowledge and developing sustainable systems.

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Abbreviations & glossary

DNN - deep neural network
GC-MS - gas chromatography coupled mass spectrometry
GC-EAD - gas chromatography coupled electroantennographic detection
MSD - mass spectrometric detector
M/Z - mass divided by charge number
VOC - volatile organic compound
Volatilome - the totality of organic volatile compounds and inorganic compounds that originates from an organism
Olfactome - all the olfactory compounds used by a particular organism
Alpha-diversity - the mean species diversity in a local system
Hill Diversity & Hill numbers - a means to calculate alpha-diversity and corresponding effective number of species within the local system
Kovàts retention index - index used to convert retention times from gas chromatography to system independent constants

1. Introduction

In order to combat the current climate change as well as securing the quality of life irrespective of where in the world one lives (Masson-Delmotte et al., 2021) the United Nations adopted the 17 sustainable development goals. The goals are a call for action to create a more sustainable world together through cooperation and united strength (United Nations, 2015). The impacts on farmers as a cause of climate change drives the current agri- and horticultural practices to practices that further accelerates climate change as well as a decline of much needed agroecosystem services creating a positive feedback loop (Mahato, 2014). The applications of pesticides to manage pests are ravaging the biodiversity of the planet, harming humans and contaminating waters and land (Aktar et al., 2009). There has to be a change from unsustainable practices to transformative and sustainable agri- and horticultural practices and methods to strengthen agroecosystems. One of the ways this can happen is through an increased cooperation between researchers as well as outlinking to the farmers' needs. Researchers need to work together and create links to society, enabling novel innovations as well as sharing data with entrepreneurs, otherwise data will sit stagnant.

1.1 Novel tools for pest control in agroecosystems

The pesticide issues In 2050 the number of humans on the planet is projected reach almost 10 billion people (United Nations Department of Economic and Social Affairs, Population Division, 2022) and with the ever increasing population of the world comes an increased demand for food for the survival of the population, and with this an intensified food production system and agricultural encroachment of nature. Almost every food production system over the world has a problem with some form of pests whether that being microbes, mammals or insects. These pests put enormous pressure on both large monocultures as well as small-scale polycultures. One of the most used pest management tools today is pesticides, that can easily be applied in various ways such as sprayed onto entire fields or as a seed coat. It has been well known that pesticides affect both plants and animals beyond their intended target (Moore, 1967). Notable are the impacts on non-target and beneficial insect species like bees or natural predators (Serrão et al., 2022), that disrupts the agroecosystem's ability to fend for themselves. Excessive usage can also make them leach into soil leading to contamination of ground water for years to come (Bhattacharyya et al., 2015), and lastly, many of the pesticides used are known to have negative effects on human health (Deguine et al., 2021). Human agricultural practices and the demand for food is driving the rapid decline of insect biodiversity (Pörtner et al., 2022; Wagner et al., 2021). Not to disturb the balances of predators and pests, biodiversity, the natural cycles of chemicals and water the crop production systems of tomorrow need to deal with pests in a way that strengthens the functionality of ecosystems while minimising risks for human health. With an improved ecological understanding of both the pests and the whole agricultural systems a more sustainable future is possible. Farmers need novel tools to manage pests and the damage that they do through sustainable pest management, but the discovery of such solutions requires a framework of cooperative innovation. In this thesis the focus is on developing leads for pest management tools using a workflow of data collection for generating leads for novel odour based attractants.

Pest management situation in Sweden As in the rest of the world, farmers in Sweden are facing hardships when problematic pesticides are being withdrawn and subsequently being banned for the damage caused to the environment and human health. An example of this is the ban on three neonicotinoids in 2013 by the European Union (Stokstad, 2018). These were banned due to the spillover effect on pollinators. This has created problems for farmers who depend on them leaving them with less options to protect their crops. For example, *Scaptomyza flava* (Meigen) is a leaf miner which targets many brassica species for oviposition and is a devastating pest for Swedish farmers leaving their crops unsellable (Alford, 1999). The Swedish Chemical Inspectorate announces on their website that the amount of trials regarding approval for use of pesticides in Sweden has gone up drastically, and with this the amount of rejections (KEMI, 2018), including pesticides which could be used to manage pests like *S. flava*.

The types of pesticide that farmers can apply to their crops are divided into categories in a few ways, for example, pesticides can be either systemic or contact based as well as be of natural or synthetic origin. Systemic pesticide application relies on the translocation through the xylem vessels of the plant. This can be done by coating the seeds or spraying it on grown plants where the pesticide is absorbed by the leaves, an application method which minimises the risk of pesticide drift off the plant and exposure to the farmer (Sanchez-Bayo et al., 2013). System pesticides are the ones getting banned forcing farmers to instead rely on the next type of pesticide. Because systemic pesticides cannot be applied to all types of seeds however, some farmers are not able to always rely on them, so sometimes they have to use non-systemic or contact based pesticides that come in contact physically with the target and the result is death by poisoning (Yadav & Devi, 2017). Contact pesticides more directly control pests, which are eliminated on contact. However, they easily run off the plant surfaces leaving only 20-30 percent of the applied pesticide left (Zhang et al., 2022). Even though these might be of natural origin, they impact the natural enemies or pollinators in the environment they are applied, examples are neem oil or pyrazhines which are acutely toxic to organisms that come in contact with them (Gandhi et al., 1988).

Introduction to the onion fly and its distribution Flies of the genus *Delia* are increasingly problematic as control possibilities available to growers are decreasing. Flies of the *Delia* genus are typically greyish brown to black in colour and oviposit and ingest on various vegetables. *Delia antiqua* (Meigen), also known as the onion fly, is a serious problem on a wide group of Allium species like yellow, red onion, leek and garlic (McKinlay, 1992). If left uncontrolled, *D. antiqua* can make the yield of mentioned crops entirely unmarketable. *Delia antiqua* is distributed throughout the temperate zone in Europe, Asia and across the northern parts of the US and Canada. Females oviposit not only on/in the target crop but also in the soil surrounding the crop, and therefore they are very hard to get rid of even with the application of pesticides on the plant as the eggs in the soil could survive (Alford, 1999; McKinlay, 1992). Other pests which are similar to *D. antiqua* also create big problems for farmers, the carrot fly *Psila rosae* (Fabricius), the cabbage root fly *Delia Radicum* (L) as well as the aforementioned the turnip leaf miner *S. flava* are similar to *D. antiqua* either in ecological niche or phenogenetically and just as with *D. antiqua* tools based on pesticides to manage these pests are disappearing due to restrictions of pesticide usage. In this thesis volatiles are mapped from host crops relevant not only to *D. antiqua* but also these species, providing valuable data beyond the scope of this thesis.

1.2 Leads for novel pest control tools

Chemical ecology Research in the field of Chemical Ecology is concerned with the identification and synthesis of the substances which carry information, with the elucidation of receptor and transduction systems which recognize and pass on these “semiochemicals”, and with the developmental, behavioural, and ecological consequences of chemical signals (ISCE, 2022). Intra- and interspecies ecological interactions among insects as well as ecological interaction between insects and other organisms are mediated to at least some degree by chemicals (Roitberg & Isman, 1992). Mating behaviour, oviposition and herbivory in crops by insect pests is partially triggered by the volatile organic compounds (VOCs) released by a crop that the insect detects and is an example of ecological response due to the detection of chemical compounds (Anderson & Anton, 2014). But insects can also use other senses in a multimodal way to orient themselves around their environment like observing their host crop with sight or other cues like for example gustation (Buehlmann et al., 2020).

Plants also have the ability to regulate their metabolic pathways as a response to stressors such as an attack of an insect pest. For example physical damage caused by an insect can upregulate certain genes, which enables an increased synthesis of certain compounds in a plant, that can ward off the attack (Brilli et al., 2019). These compounds could in turn be attractants for a natural predator of the herbivore which could predate and kill off the pest, or an antifeedant or toxic compound which lowers the impact of the herbivore (Dicke, 2009).

Methods for mapping volatile organic compounds and identifying electrophysiological responses Techniques utilising gas chromatography have been around since the introduction of gas-liquid partition chromatograms for analytical purposes by Martin and Synge in 1941 (Martin & Synge, 1941). A modern capillary gas chromatograph operates in the following way: a carrier gas, like helium or hydrogen, is supplied to a heated inlet, where a liquid sample is injected and instantly volatilised; the sample is then carried into the column by the carrier gas, where it is separated into individual compounds based on the compounds affinity with the column’s stationary phase (Fig. 1). The column can for example separate compounds depending on the molecules polar properties and/or their size. The columns are usually between 5-30 metres long and have varying diameters ranging in less than 1 mm thickness. The column resides inside of an oven which often starts at a lower temperature of around 30 °C and can ramp up to temperatures of up to 400, depending on the column used.

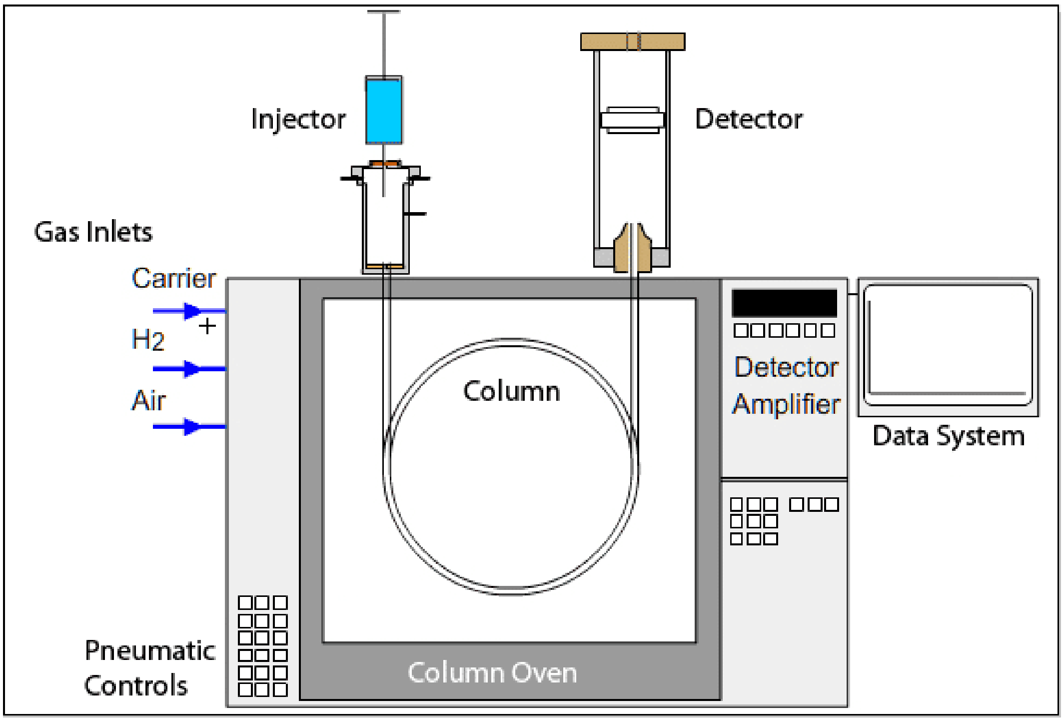

Figure 1: Schematic of a gas chromatograph complete with gas inlet, injection inlet, column, detector, amplifier and connected data system. Sample is injected into the injector (blue cylinder) which is passed down with a carrier gas to the column which resides in the column oven where the sample is heated and sent to the detector and further the amplifier and data is then sent to the data system.

A range of detectors can be coupled to the gas chromatograph; two are used in this thesis: the mass spectrometry detector (MSD) and the flame ionisation detector (FID). The mass spectrometer is useful for identifying compounds. This is done using an electrical charge that ionises the molecule. The resulting ions produce a spectra of m/z thus allowing the chemical compound to be identified. (Chromacademy, n.d.; Evers, 2014). The FID is a detector which pyrolyses the eluted sample with a hydrogen flame ionising the compounds in the sample, and does not produce a spectra that can be used to identify the compound. The ions are then repelled towards collector plates which detect the ions creating a signal by measuring the current produced by the ions which is sent to an amplifier and forwarded to a computer system (Skoog et al., 2017).

In this thesis we utilise a setup where a GC is coupled to a rig set-up for electroantennographic detection (EAD), the setup consists of an immobilised insect mounted with electrodes on an olfactory organ. GC-EAD can be used for distinguishing what compounds present in a mixture of volatiles stimulate the olfactory receptors of insects. Changes of voltage across the antennae of insects are measured when exposed to the volatile compounds which are eluted by the GC giving responses in the form of a chromatogram. These compounds can be either volatile semiochemicals released by plants or other insects as well as pheromones (Slone & Sullivan, 2007).

Linking olfactomics with databases and the implementation of R packages for data analysis By mapping the volatile profile (volatilome) of a certain crop using a gas chromatography coupled mass spectrometer (GC-MS) system it is possible to identify compounds that make up the headspace. Such a volatilome can then be separated into its individual components and be passed over the olfactory organ of an insect pest using GC-EAD giving a detailed picture of what compounds the insect can detect. By understanding what compounds that are detected, attractive blends can be deduced through laboratory or field trials. The result can be novel pest management tools that are target pest-specific (Larsson Herrera et al., 2020). Such lures can be used to attract the pests to pesticide-laced lures, a contrast to non-specific pesticides that are sprayed across the field killing much of which has the misfortune to make contact. Such lures can work in tandem with agroecological practices minimising exposure to humans, animals and the environment benefiting both biodiversity and the field workers. A targeted usage of pesticides, along with reduced machinery usage also leads to less emissions of greenhouse gases creating sustainable advantages compared to traditional methods (Navarro-Llopis et al., 2013; Radcliffe et al., 2008). To be able to accelerate the development of such lures, research needs to be transparent and reproducible.

Over decades, a large number of EAD-studies have been published, However, unfortunately they lack a standardisation, and rigid analyses through comparative approaches. For instance, many published papers lack retention indices or quantitative response data making it hard to replicate the experiments. However, if the produced data was standardised it would be possible to comparatively analyse huge amounts of EAD-related papers. The results would hence be drawn from earlier research and make it possible for researchers to analyse their data in the context of other insects and volatile sources. There is a need to have a standardised way of working with GC-EAD data during the gathering, manipulation and analysis phases. Many lessons can be drawn from the field of genomic data where standardisation and data sharing is prevalent (Roy et al., 2018). In this thesis we have utilised a GC-EAD workflow (olfactomics) for a problematic pest species, *D. antiqua*, that makes it possible to have a standardised way of analysing GC-EAD data and branched the workflow out to additional open access databases and packages.

1.3 Aims and objectives

The aim of this study was to map the volatilome of a set of host crops, identify the olfactome of *D. antiqua* and comparatively compare what *D. antiqua* senses in the headspace with *D. melanogaster* of these selected crops, including the target crop onion. Furthermore, the aim is to adhere to a standardised and digitised workflow for olfactomics data analysis.

Objectives for the study were as follows:

1. To map the volatilome of different crops through GC-MS.
2. To annotate and identify what specific compounds within these volatilomes which elicit electrophysiological activity in flies through GC-EAD.
3. To adhere to a standardised workflow for MS and EAD data analysis integrating packages and databases in R.

2. Material and methods

2.1 Insect rearing

Pupae of *Delia antiqua* (Diptera: Anthomyiidae) were obtained from De Groene Vlieg, Dronten, the Netherlands. This species was reared in a confined room at room temperature in polyester netting bugdorm insect cages (325 x 325 x 325 mm). The flies were fed a diet consisting of 1:1:1 of white sugar (Dan Sukker, "Svenskt Strösocker", Nordic Sugar, Malmö, Sweden), creamer powder (AH, "Koffiecreamer", Zandaam, The Netherlands) and bakers yeast *Saccharomyces cerevisiae* (Meyen). The flies also had access to a 1:1 mixture of honey and water as well as cotton balls soaked with tap water. Everything was replaced every second day. As pupae emerged over many days, the pupae were moved to a new cage every 3 days to create cohorts of three day old flies.

Drosophila melanogaster of the Dalby strain were kindly provided by Charles Kwadha in SLU Alnarp. *Drosophila melanogaster* was kept in plastic tubes and flipped twice a week into new tubes for oviposition. Each tube contained a feed consisting of water, corn meal, plant agar, malt, bakers yeast (*S. cerevisiae*), soy meal, sugar syrup and propionic acid.

2.2 Volatile collection, processing and analysis

Headspace collections Volatiles from yellow onion, napa cabbage, carrot and radish were collected prior to the experiments. These were collected using an open-looped filtration system. A pump circulated air firstly through a carbon filter for purification and led through a polyester bag (Toppits, Minden, Germany) which contained the sample for 4 hours. The air was then filtered through a teflon tube containing a 50 mg Porapak-Q filter (Markes International, Llantrisant, United Kingdom). Airflow through the filtration system was 1 l/min. The released volatiles were adsorbed to the Porapak-Q beads which were eluted from the filters using 300 µL n-hexane as solvent into 1.5 mL glass vials. There were a series of 4 samples for each crop which were combined into one. The mixes were left in a fume hood to concentrate until approximately half of the original solvent remained, thus increasing concentration of the samples to be used for injections in both GC-MS and GC-EAD.

Gas chromatography coupled mass spectrometry Collected volatiles were analysed using two separate setups of GC-MS. One machine was an Agilent Technologies 7890B coupled with 5977A MSD which was lined with a DB-WAX column (60 m x 250 µm x 0.25 µm), with helium as the carrier gas. Samples were injected into a 250 °C splitless injection port using an Agilent Technologies 7650A Automatic Liquid Sampler. The GC oven was programmed as follows: initial oven temperature was set at 40 °C and held for 3 minutes, then the temperature ramps with 8 °C per minute up to 240 °C and is then held for 25 minutes. Finally a post run at 250 °C was run for 1 minute to clean the column from any eventual leftover sample.

The program and hold time for DB-WAX was intentionally longer than necessary to assure that all compounds had been eluted from the column both to make sure that all data was gathered as well as allowing for creating a smarter program for the GC in the EAD system.

The other machine was an Agilent Technologies 6890N coupled with 5975 MSD lined with a HP5-MS column (60 m x 250 µm x 0.25 µm) using helium as carrier gas. Samples were injected into a 250 °C splitless injection port using an Agilent Technologies 7683B Automatic Liquid Sampler. The GC oven was programmed as follows: initial oven temperature 40 °C held for 3 minutes, then ramp starts 8 °C per minute up to 275 °C and then held for 10 minutes. No post run.

Gas chromatography coupled electroantennographic detection GC-EAD recordings were done on female individuals who were between 5 - 14 days old with a preference of individuals not being older than 14 days. An Agilent Technologies 7890A gas chromatograph with DB-WAX columns (30 m x 0.25 mm thickness, film thickness 0,25 µm column) was coupled with the EAD set-up. The GC oven was programmed as follows: initial oven temperature was set at 30 °C and held for 3 minutes, then the temperature ramps with 8 °C per minute up to 225 °C and is then held for 13 minutes. Finally a post run at 250 °C was run for 1 minute. With the column being split, half of the volatiles went to the flame ionisation detector and half passed over the insect's olfactory organ, in this case the antennae. Helium was used as the carrier gas. The effluent was passed over the fly which was constrained in a pipette tip. A reference glass capillary was inserted into the head of the fly and a recording glass capillary was connected to the tip of the fly antennae. Both glass capillaries were filled with Beadle-Ephrussi ringer solution. A GC-EAD software (Syntech GcEad 2014, Ockenfels Syntech, the Netherlands) was used to record EAD and FID signals.

To determine responsiveness of the mounted fly a puff of isoamyl acetate at a concentration of 10 ng/µl, mixed in n-hexane, was puffed, using an airflow of 1.5 l/minute. The fly was deemed as responsive if it elicited a response and GC-EAD was performed. For each insect species at least 3 replicates were recorded.

Synthetics and blend To confirm that the compounds that elicited electrophysiological responses were correctly identified synthetic equivalents was necessary. The following synthetic compounds were tested: allyl methyl disulphide (2179-58-0), dipropyl trisulphide (6028-61-1), dipropyl disulphide (629-19-6), allyl propyl disulphide (2179-59-1) and methyl propyl disulphide (2179-60-4). These compounds were diluted in n-hexane in a series from neat to 0.01 mg/µl to 100 ng/µl to a final concentration of 10 ng/µl. A mixture of the compounds was then injected using the GC-EAD. Compounds which were not available in the chemical storage at the department of SLU Alnarp were purchased from chemtronica.com.

A standard blend of n-alkanes, ranging from heptane up to triacontane, was injected on both GC-MS and GC-EAD; this was done to have a standard set of compounds with known retention times to compare the unknown compounds from the samples with.

2.3 Data analysis

The data generated from GC-MS were analysed with MassHunter Qualitative Navigator B.08.00 (Agilent Technologies, USA). Peaks were extracted, automatically integrated (with a threshold of 10000) and then tentatively identified with matching spectras in MS Search 2.4 (NIST, Gaithersburg, USA) using MS libraries Wiley12 and NIST20. The tentative results were then additionally supported by comparing the calculated Kovàts retention indices with published ones and finally confirmed using synthetic standards. Kovàts helps to convert the fairly uncertain nature of retention times into system-independent constants. Some compounds were annotated as contaminants/trace elements. These compounds were deemed contaminants/trace elements if RI, structure or m/z was not matching due to the quantity of the compound in the sample being too small to identify, or if the annotation contained siloxane.

Annotation of EAD peaks was done using Syntech 2014. Through visual annotation and quantitative marking of the EAD peaks a millivolt value was given, indicating the responsiveness to a certain compound. These compounds could be identified tentatively by comparing the Kovàts retention indices (RI) from GC-MS and matching these RI with the FID peaks from the GC-EAD experiment, if the RI as well as peak shapes of a response corresponded to the RI of a compound in the MS RI it was then tentatively identified. The EAD traces were normalised as per the method in (Biasazin et al., 2019).

As a part of the workflow, data from the MS as well as the EAD was all annotated into a standardised spreadsheet that also calculated Kovàts retention indices given a set of n-alkanes. This was done to assure the quality of the collected data. The standardised and filled out spreadsheets could then be then imported into R (R Core Team, 2021) using custom written functions that utilised the ability of the openxlsx package (Schauberger & Walker, 2021) to read xlsx files. The resulting compounds were then used to construct a database that was further annotated and checked for doubles by retrieving the Standard InCHI key through the “Chemical Identifier Resolver” from Cactus (Peach & Nicklaus, 2018). The standardisation in annotation combined with InChi allowed for integration of NPClassifier (Kim et al., 2021) to deduce molecular pathways as well as functional groups. It also allowed for integration with the package Chemodiv (Petrén et al., 2022) for constructing molecular networks and dissimilarity indices. Hierarchical clustering was then performed on the resulting dissimilarity indices allowing for the construction of dendrograms, the dendrograms were then plotted using ggtree (Yu et al., 2017). Heatmaps were produced using ggplot, and molecular networks were drawn using a modified version of functions within Chemodiv. All data manipulation was done using the tidyverse (Wickham et al., 2019). The table showing the chemical compounds tentatively identified from analysis was created using xtable (Dahl et al., 2019) and LaTeX.

3. Results

Volatiles released from yellow onion, napa cabbage, carrot and radish contained 162 tentatively identified compounds in total, excluding likely contaminants/trace elements. These compounds belonged to various functional classes like aldehydes, aromatic solvents, esters fatty alcohols, hydrocarbons, organic di- and trisulphides, and terpenoids. The composition of the different crop headspaces varied quite drastically. While all samples contained compounds from different functional classes like aromatic solvents, fatty alcohols and hydrocarbons not all samples contained all different functional classes. Yellow onion and napa cabbage for example were the only ones that contained sulphuric compounds, in the form of dialkyl disulphides and organic di- and trisulphides. In yellow onion 12 of the 51 (24%) tentatively identified compounds contained sulphur and in napa cabbage 4 of the total 27 compounds (15%). Comparatively neither the radish nor the carrot headspace contained any sulphuric compounds. Radish contained 19 identifiable compounds in total and carrot 60 identifiable compounds. The radish headspace was made up of hydrocarbons and terpenoids as well as one ester. The carrot headspace was made up overwhelmingly by different kinds of terpenoids, a few fatty alcohols and hydrocarbons, 27 out of the 60 compounds were terpenoids.

3.1 Headspace analysis, chemical diversity and molecular networks

There was a stark difference in composition of compounds and the quantity of compounds between the headspace samples. There was some overlap between the samples. For example, all samples contained the pinane terpene α-Pinene, the aromatic solvent p-Xylene and the monocyclic monoterpenoid D-Limonene. There were some additional overlaps, three of the four samples contained compounds like nonane, undecane, diacetone alcohol and mesitylene (Fig. 2). When comparing both the quantity of compounds across the samples as well as the abundance (the area of the peak from MS) of these samples there were some differences. The carrot sample contained the most compounds (38% of all compounds including overlap) and they were low in abundance except for myrcene which had high abundance. The general abundance of all the compounds, including compounds which were of lower abundance, across all samples can be seen in Fig. 2. The dendrogram tree in Fig. 2. shows the relatedness between different compounds based on their chemical diversity. There were a few compounds that were outliers in regard to their molecular structure. These compounds can be found in the bottom of the heatmap and respectively dendrogram tree (Fig. 2). The most obvious outliers were two fatty acid compounds containing chlorine. Another outlier was the only pyrazine in the dataset, 5-(sec-Butyl)-2,3-dimethylpyrazine. To identify peaks from GC-MS, experimental Kovàts retention indices (RI) from GC-MS were compared with the closest published values from literature. Table 1 contains the compounds, CAS and associated Kovàts retention indices from GC-MS.

Using the chemodiv package molecular similarity was calculated and allowed for visualisation of the relationship between molecules in the form of a molecular network (Fig. 3) as well as the dendrogram in Fig. 2. The deep neural network (DNN) based tool, NPClassifier, allowed for annotation of the metabolic pathways for the tentatively identified compounds. Six pathways could be identified from the compounds: alkaloids, amino acids and peptides, fatty acids, polyketides, shikimates and phenylpropanoids and terpenoids with the pathway of some compounds left not identifiable (NA). The cluster of both larger and smaller orange dots in the molecular network (Fig. 3) shows the many kinds of terpenoids in the carrot sample, in total 73% of the total amount of terpenoids across all samples were from carrot. Yellow onion and napa cabbage samples contained compared to carrot fewer terpenoids and contained a unique subset of compounds. These are represented in the molecular network as filled in grey dots which are compounds which have a non identifiable molecular pathway with the NPClassifier database. These were the sulphuric based compounds unique to these two samples. Using the database NPClassifier and the chemodiv package it was possible to calculate the alpha-diversity for the four vegetable samples. Calculating the Hill Diversity and attached Hill numbers or the effective number of species shows that carrot had 17.54, yellow onion had 13.32, napa cabbage had 9.85 and radish 7.33 “species” of compounds in each respective sample. This shows that carrot had the highest amount of different “species” of chemical compounds and radish had the lowest.

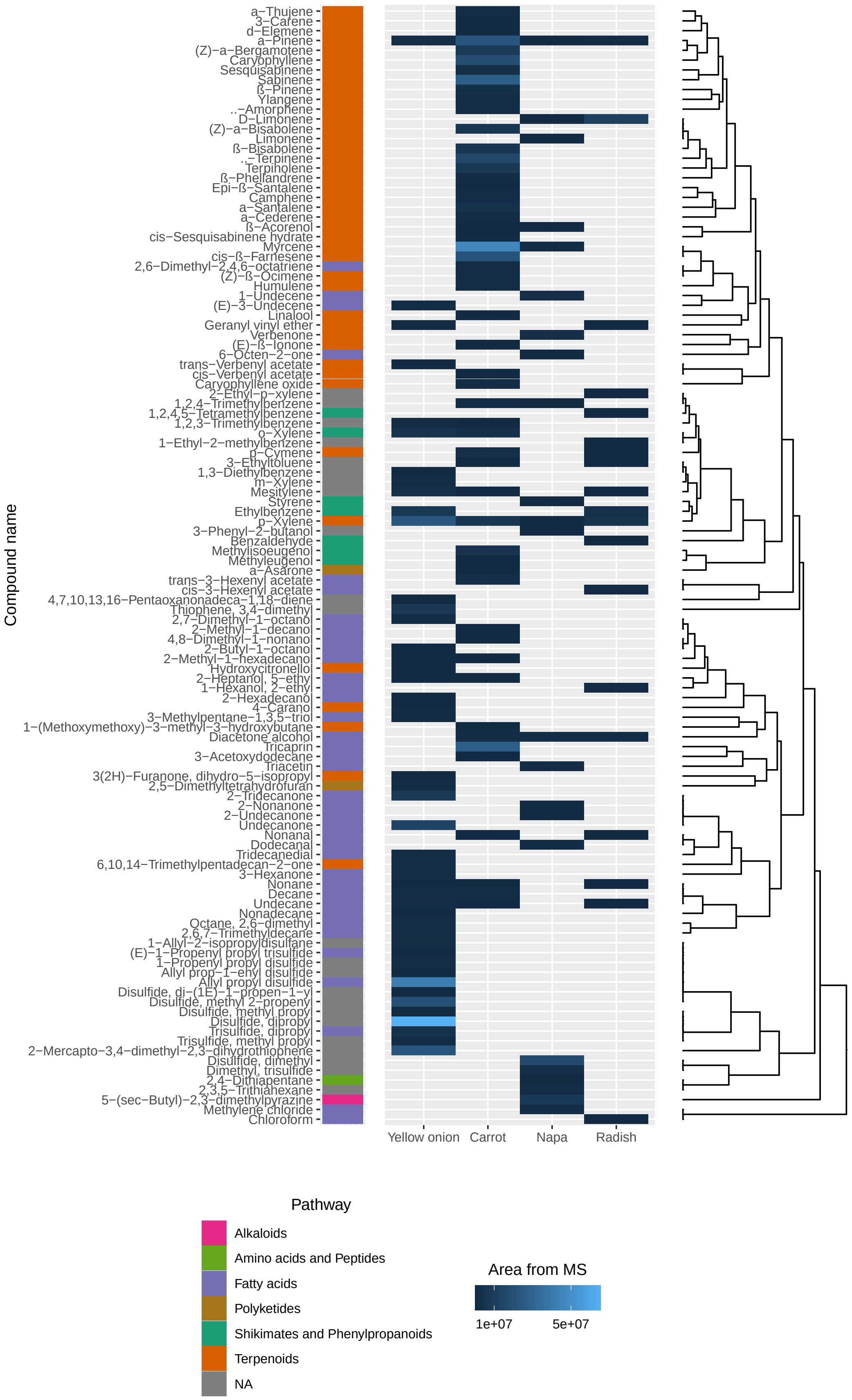
Compound name
a−Thujene
3−Carene
d−Elemene
a−Pinene
(Z)−a−Bergamotene
Caryophyllene
Sesquisabinene
Sabinene
ß−Pinene
Ylangene
..−Amorphene
D−Limonene
(Z)−a−Bisabolene
Limonene
ß−Bisabolene
..−Terpinene
Terpinolene
ß−Phellandrene
Epi−ß−Santalene
Camphene
a−Santalene
a−Cederene
ß−Acorenol
cis−Sesquisabinene hydrate
Myrcene
cis−ß−Farnesene
2,6−Dimethyl−2,4,6−octatriene
(Z)−ß−Ocimene
Humulene
1−Undecene
(E)−3−Undecene
Linalool
Geranyl vinyl ether
Verbenone
(E)−ß−Ionone
6−Octen−2−one
trans−Verbenyl acetate
cis−Verbenyl acetate
Caryophyllene oxide
2−Ethyl−p−xylene
1,2,4−Trimethylbenzene
1,2,4,5−Tetramethylbenzene
1,2,3−Trimethylbenzene
o−Xylene
1−Ethyl−2−methylbenzene
p−Cymene
3−Ethyltoluene
1,3−Diethylbenzene
m−Xylene
Mesitylene
Styrene
Ethylbenzene
p−Xylene
3−Phenyl−2−butanol
Benzaldehyde
Methylisoeugenol
Methyleugenol
a−Asarone
trans−3−Hexenyl acetate
cis−3−Hexenyl acetate
4,7,10,13,16−Pentaoxanonadeca−1,18−diene
Thiophene, 3,4−dimethyl
2,7−Dimethyl−1−octanol
2−Methyl−1−decanol
4,8−Dimethyl−1−nonanol
2−Butyl−1−octanol
2−Methyl−1−hexadecanol
Hydroxycitronellol
2−Heptanol, 5−ethyl
1−Hexanol, 2−ethyl
2−Hexadecanol
4−Caranol
3−Methylpentane−1,3,5−triol
1−(Methoxymethoxy)−3−methyl−3−hydroxybutane
Diacetone alcohol
Tricaprin
3−Acetoxydodecane
Triacetin
3(2H)−Furanone, dihydro−5−isopropyl
2,5−Dimethyltetrahydrofuran
2−Tridecanone
2−Nonanone
2−Undecanone
Undecanone
Nonanal
Dodecanal
Tridecanedial
6,10,14−Trimethylpentadecan−2−one
3−Hexanone
Nonane
Decane
Undecane
Nonadecane
Octane, 2,6−dimethyl
2,6,7−Trimethyldecane
1−Allyl−2−isopropyldisulfane
(E)−1−Propenyl propyl trisulfide
1−Propenyl propyl disulfide
Allyl prop−1−enyl disulfide
Allyl propyl disulfide
Disulfide, di−(1E)−1−propen−1−yl
Disulfide, methyl 2−propenyl
Disulfide, methyl propyl
Disulfide, dipropyl
Trisulfide, dipropyl
Trisulfide, methyl propyl
2−Mercapto−3,4−dimethyl−2,3−dihydrothiophene
Disulfide, dimethyl
Dimethyl, trisulfide
2,4−Dithiapentane
2,3,5−Trithiahexane
5−(sec−Butyl)−2,3−dimethylpyrazine
Methylene chloride
Chloroform
Yellow onion
Carrot
Napa
Radish
Pathway
Alkaloids
Amino acids and Peptides
Fatty acids
Polyketides
Shikimates and Phenylpropanoids
Terpenoids
NA
Area from MS
1e+07
5e+07

Figure 2: Heat map based on the data generated from GC-MS. The figure shows (left to right) compound name found across all samples, metabolic pathway of the compound, the heat map itself with the presence and abundance of the compounds in the individual samples, a dendrogram showing relatedness between the compounds. At the bottom there is a legend for the metabolic pathways of the compounds as well as a legend for ratio of abundance as the area of the peaks from MS going from 10 million to 50 million. The compounds are arranged in order of the calculations of relatedness of the compounds. The metabolic pathways are based on the NPClassifier DNN database and include alkaloids (pink), amino acids and peptides (light green), fatty acids (purple), polyketides (brown), shikimates and phenylpropanoids (emerald green), terpenoids (orange) and lastly NAs (grey).

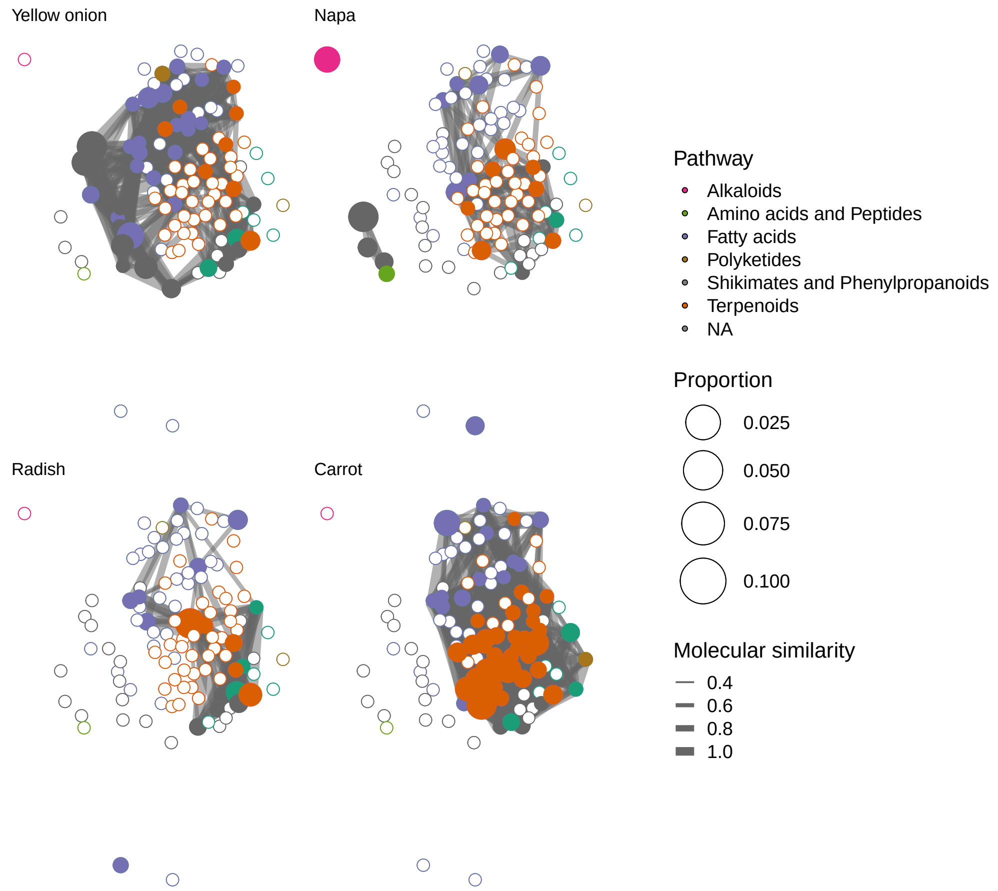

Figure 3: A molecular network of volatiles from headspace samples. The figure contains four representations of the molecular network built on the total amount of identified compounds across the four headspace samples. There are three parts to the legend. The metabolic pathway which just as in the last figure is based on the NPClassifier DNN and each pathway is represented as a colour. These are as follow: alkaloids (pink), amino acids and peptides (light green), fatty acids (purple), polyketides (brown), shikimates and phenylpropanoids (emerald green), terpenoids (orange) and lastly NAs (grey). The size of the dot is represented as the proportion concentration of the compound in the sample and lastly the molecular similarity is represented by the thickness of the bridging lines. The networks are arranged as follows: yellow onion, napa cabbage, radish and carrot. Each compound is represented as a dot in the network, if the dot is filled in with a colour it is present in the sample and if not it is white.

3.2 Similarities and differences in electrophysiological responses between *D. antiqua* & *D. melanogaster*

Through GC-EAD recordings the antennae of the two dipteran species tested, *D. antiqua* and *D. melanogaster*, were exposed to the different headspace samples. In total 34 compounds elicited electrophysiological responses in the antennae in either *D. antiqua* or *D. melanogaster*. A heatmap of these responses along with compounds, the functional class from NPClassifier and a legend of normalised response was made (see Fig. 4). Furthermore, traces for *D. antiqua* printed to visualise the responses as they were recorded (see Fig. 5) as well as for the synthetic blend on both species (Fig. 6) The responses show that only one compound across the four samples had a response in both species, namely 3-Hexen-1-ol, (E). The remaining 33 compounds had a response only in either *D. antiqua* or *D. melanogaster*. *Delia antiqua* responded to a total of 29 compounds in the samples and *D. melanogaster* responded to 6 compounds. The responses did not highlight any prevalence of homogeneity in functional classes with the exception that *D. antiqua* responded to several sulphuric compounds. *Drosophila melanogaster* responded to at least one compound in all samples except for yellow onion, where none were detected, and not to a single compound containing sulphur. Ten of the compounds were unidentifiable and have been labelled as UnknownX_Sample. As a means to further confirm the responses from the GC-EAD results, the antennae of the dipteran species were exposed to a mix of the sulphuric compounds in the headspace at a concentration of 10 ng/µl dilution. *D. melanogaster* did not respond to any of the sulphuric compounds in the synthetic mix while *D. antiqua* responded to 3 of them, namely allyl propyl disulphide, dipropyl disulphide and dipropyl trisulphide. The same which was found in the volatilome of yellow onion. This supports the identification of the sulphur containing compounds that elicited a response in *D. antiqua* as can be seen in Fig. 4.

Retention indices for the tentatively identified compounds derived from the EAD responses can be found in table 1. The compound name along with the CAS-number as well as which sample the compound is part of is presented in the first three rows. As well as the RI from MS, the mean closest RI from published articles and lastly RI from EAD from either *D. antiqua* and *D. melanogaster*.

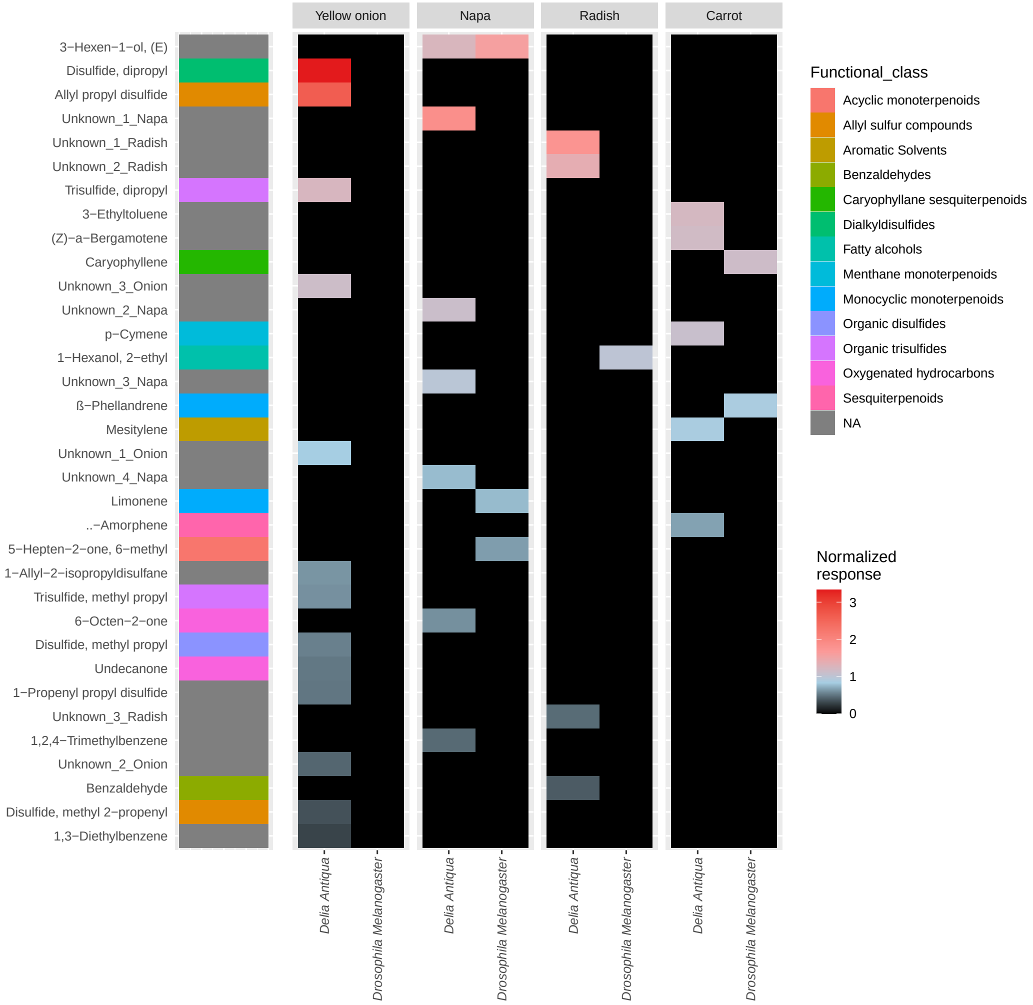

Figure 4: Heat map showing the electrophysiological responses to headspace samples in *D. antiqua* & *D. melanogaster*. The figure shows (from left to right) Compound name, Functional class of compound, The heatmap itself displaying normalised responses from the antennae with black showing no response (black). On top of the heatmap, the sample name is indicated: yellow onion, napa cabbage, radish and carrot. On the bottom of the heatmap the insect species are presented. To the most right there are two legends, one showing the functional classes from NPClassifier (NP) & Pubchem (P) containing acyclic monoterpenoids (NP), allyl sulphur compounds (P), aromatic solvents (P), benzaldehydes (P), bergamotane sesquiterpenoids (NP), dialkylsulphides (P), fatty alcohols (NP), menthane monoterpenoids (NP), monocyclic monoterpenoids (NP), organic disulphides (P), organic trisulphides (P), oxygenated hydrocarbons (NP), sesquiterpenoids (NP) and lastly NA which were compounds non-classifiable by either NP or P. The other part of the legend is the normalised electrophysiological response.

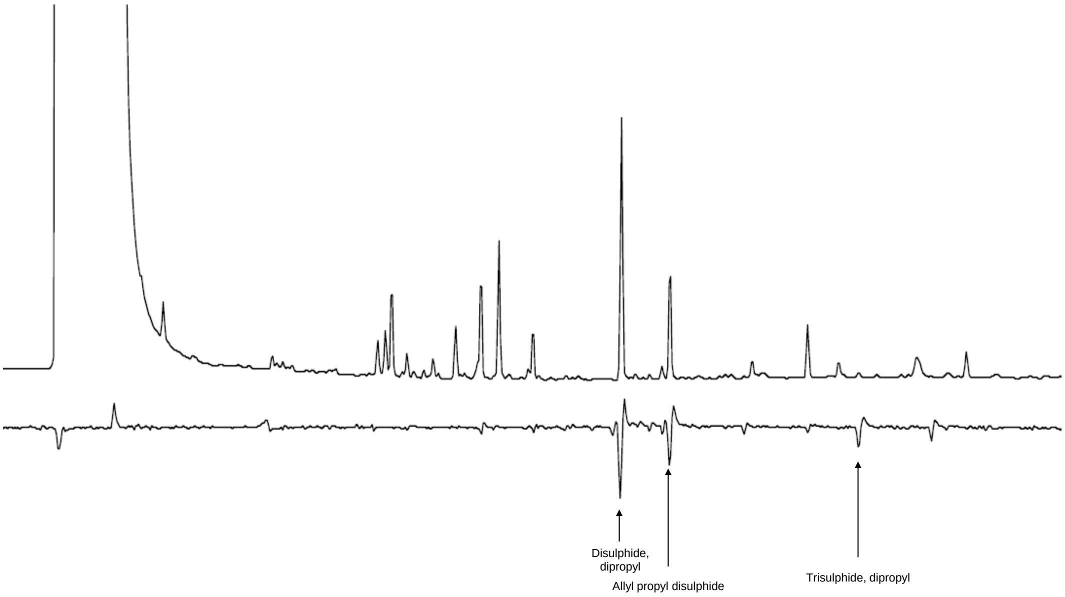

Figure 5: Traces showing the FID peaks with the EAD-peaks from the eluted compounds in tandem for yellow onion on *D. antiqua*.

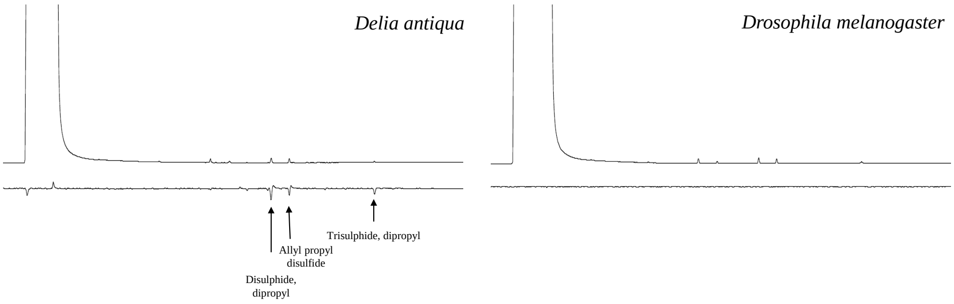

Figure 6: Traces showing the FID peaks with the EAD-peaks from the eluted compounds of the synthetic blend in tandem on *D. antiqua* (left) and *D. melanogaster* (right).

Table 1: Table containing compound names, CAS-numbers as well as retention indices from MS, EAD and closest published values, $*$ Compounds confirmed with synthetics

Compound Name	CAS	Sample name	MS DB-WAX	Mean DB-WAX	MS HP5-MS	Mean HP5-MS	*D. Antiqua*	*D. melanogaster*
Unknown_1_Radish		Radish			1.12			
Unknown_1_Napa		Napa			1.13			
Unknown_1_Onion		Yellow onion			1.13			
β-Phellandrene	555-10-2	Carrot	1202.19	1207.00				1203.56
Limonene	138-86-3	Napa	1203.53	1198.00	1030.3	1030	1203.61	
Disulphide, methyl propyl	2179-60-4	Yellow onion	1222.76	1229.00	933	932.1	1220.35	
3-Ethyltoluene	620-14-4	Carrot	1234.37	1222.00			1233.38	
Mesitylene	108-67-8	Carrot	1251.79	1250.00	970.8	975	1249.44	
p-Cymene	99-87-6	Carrot	1261.14	1267.00			1162.97	
1,2,4-Trimethylbenzene	95-63-6	Napa	1272.74	1278.00			1269.46	
Disulphide, methyl 2-propenyl	2179-58-0	Yellow onion	1278.10	1276.00			1275.27	
1,3-Diethylbenzene	141-93-5	Yellow onion	1301.87		1051.5	1056	1305.26	
6-Octen-2-one	35194-31-1	Napa	1318.38				1315.66	
5-Hepten-2-one, 6-methyl	110-93-0	Napa	1324.07	1339.50				1327.44
3-Hexen-1-ol, (E)	928-97-2	Napa	1364.09	1367.00			1363.44	1364.88
Disulphide, dipropyl*	629-19-6	Yellow onion	1374.42	1377.50	1108.8	1098	1368.81	
1-Propenyl propyl disulphide	23838-20-2	Yellow onion	1406.52	1406.00	1117.6	1117.1	1402.70	
1-Allyl-2-isopropyldisulphane	67421-85-6	Yellow onion	1421.01				1418.77	
Allyl propyl disulphide*	2179-59-1	Yellow onion	1430.52	1428.00			1426.28	
1-Hexanol, 2-ethyl	104-76-7	Radish	1469.46	1491.00				1464.14
Benzaldehyde	100-52-7	Radish	1508.53	1516.00			1508.75	
Trisulphide, methyl propyl	17619-36-2	Yellow onion	1526.08	1529.00			1516.19	
(Z)-α-Bergamotene	18252-46-5	Carrot	1585.61	1564.50	1414	1414	1593.67	
Undecanone	112-12-9	Yellow onion	1589.38	1598.00			1593.04	
Caryophyllene	87-44-5	Carrot	1605.59	1594.00	1441	1418		1601.75
Unknown_2_Onion		Yellow onion			1647.50			
Trisulphide, dipropyl*	6028-61-1	Yellow onion	1670.61	1672.00	1335	1337.3	1659.43	
Unknown_2_Napa		Napa			1683.90			
Unknown_2_Radish		Radish			1711.91			
γ-Amorphene	6980-46-7	Carrot	1715.99	1708.00			1707.39	
Unknown_3_Onion		Yellow onion			1759.43			
Unknown_3_Radish		Radish			1794.70			
Unknown_3_Napa		Napa			2406.76			
Unknown_4_Napa		Napa			2455.78			

4. Discussion

The publish or perish paradox is an idea that researchers are forced to produce quantities of papers putting enormous pressure on researchers (Moosa, 2018; Rawat & Meena, 2014). Each year about 1.8 million papers is published in circa 28.000 journals (Ware & Mabe, 2012). The process of doing research in the field of chemical ecology and olfactomics could be beneficial and streamlined so that the fundamental research leads to novel pest management tools through sharing of data, knowledge and thoughts throughout the whole chain and not just to pump out results for increased impact factors in journals. The need of empowering researchers with databases, analytical tools and support results to be comparable is crucial and in this thesis we utilise a comparative approach, olfactomics, to generate data in a standardised format to ensure as much transparency as possible as well as having a workflow available for other researchers to replicate the results. These results could then move from fundamental science to entrepreneurs who have the skillset of creating ideas and finding innovators to come up with the solutions farmers need. Utilising the high throughput system of GC-EAD, standardised data sheets, open access databases as well as programming packages we have tried to create a standardised workflow in olfactomics which have generated data for the development of novel lures for the management of *D. antiqua* as well as showing how to utilise GC-EAD to create high-throughput systems beyond pheromones in a standardised way.

4.1 Tentative identification, unidentifiable compounds and chemical analysis

A category of chemical compounds which have been excluded from the figures and most of the analysis are the compounds which were deemed too small to identify or contaminations while analysing the data. These compounds made up 66% of all headspace sample compounds but were disregarded and not included in the results, figures and tables. These compounds which were too small to be identified were deemed as such because the equipment did not give a high enough resolution output. There were also a group of compounds which were deemed contaminants containing siloxane which make up part of the columns used inside the GC and have bled into the sample. Furthermore, there are other contaminants that do not contain siloxane, such contaminants could have contaminated the samples from many sources such as equipment, laboratory surfaces to glove or just the environment in which the headspace samples were collected (Tsikas, 2010) or be trace elements which could not be assertively identified. The compounds which are not containing siloxane are harder to deem simply as contaminations but not impossible due to the inability to identify them.

There were certain types of compounds which were deemed contaminations due to the fact that they were present across all samples in many places in each sample as well as in the synthetic blend where only the 5 compounds which make up the blend should be present. While there should only be 5 peaks for each compound and a peak for the solvent there were 50 peaks total in the synthetic blend, meaning that 44 of these compounds are probable contaminants. These contaminations were typically made up of long chains of carbon and oxygen or rings of carbon and oxygen as well, and these were appearing in different parts of the headspace sample independent to a specific time in the elution of the GC. Some of the compounds which were identifiable were detected in all samples like the aromatic solvents p-Xylene and mesitylene. These are probably contaminations from somewhere during the lab work and handling of samples but it is almost impossible to say, but as they appear in every sample it is probable.

Due to the lack of comparable data in the training set for the neural network there are some complications in the chemical analysis as NPClassifier have a hard time identifying the pathways and functional classes of some compounds. These are the ones which are labelled with a grey or NA functional class in Fig. 2 and the grey dots in the molecular network (Fig. 3). It is stated in the NPClassifier article that the neural network is not yet able to identify the pathway and functional class of every chemical compound there is (Kim et al., 2021) which can be observed here. This has to do with the rarity of some compounds compared to others and as the authors present it, deficiency in training of the neural network. Where it was not possible for NPClassifier, the functional class has been identified instead with either ClassyFire (Djoumbou Feunang et al., 2016) or the PubChem library of chemical compounds. This creates some inconsistency in the classification of compounds and will be sorted as the neural network underlying NPClassifier is further trained.

The four different samples presented a varied composition in quantity, as well as quality, of compounds in the respective volatilomes. The volatilomes of the samples are both the identifiable and non-identifiable compounds. Onion contained 151 compounds, carrot 133, napa cabbage 131 and radish 120. Of the 151 compounds in the onion sample only 11.7% of the total compounds, elicited responses in *D. antiqua* but could be attributed to 53% of the total area from GC-MS. This is noticeably different to the other headspace samples where the compounds that elicited responses did not cover more than 6.5% of the total area. In radish compounds that elicited a response could only be attributed to 0.5% of the total area.

Comparing the chemical composition of the different crops is quite interesting as they originate represent a wide phylogenetic diversity: napa cabbage and radish are both brassicas, yellow onion is an allium and carrot is from the daucus genus. The chemical composition in yellow onion and napa cabbage had a lot of overlap as well as napa cabbage and radish while carrot was clearly different. So despite napa cabbage and yellow onion being further related than napa cabbage and radish they might still share some similarities in their metabolic pathways due to having genes which express the synthesis of the same compounds and produce more similar chemical compounds than napa cabbage and radish. Out of all of these compounds only a selected few elicited responses in the flies and while all crops elicited responses in both flies (with the exception of yellow onion on *D. melanogaster*) there were clearly more responses to volatiles from yellow onion and napa cabbage in *D. antiqua*.

4.2 Phylogenetic divergence of *Drosophila* to *Delia* and sulphuric compounds in nature

The results from GC-EAD revealed the complete lack of detection in *D. melanogaster* of the sulphur based compounds found in the tentative volatilomes of yellow onion and napa cabbage, using both headspace collected samples and synthetics. On the other hand, did *D. melanogaster* detect compounds which *D. antiqua* did not. There is a clear difference in detection patterns between the two species that are separated by both evolutionary and ecological distances. Some of the compounds which elicit electrophysiological responses in *D. antiqua* are partially ones containing sulphur. Comparing then to *D. melanogaster* which on the other hand has a preference to different vinegar compounds like acetic acid (Becher et al., 2010) and compounds released from overripe fruit like mango and plum (Zhu et al., 2003). Sulphuric compounds are often part of volatilomes of highly organoleptic nature that have very distinct and pungent smells and flavours (Woker, 2002). These compounds can be from many different origins in nature, such as decaying meat, carnivore dung and rotten eggs (McGorrin, 2011; Moré et al., 2013), but also in plants such as brassicas and alliums as well as certain flowers which produce oligosulphides as attractants of prey (Lanzotti et al., 2014; Liu et al., 2018). Sulphuric compounds can also be of inorganic origin and be found in places like hot-springs and volcanoes (Brasted, 2022; Moré et al., 2013). This means that at some point in time the Anthomyiidae family or only *D. antiqua* might have gained sensitivity towards sulphur containing compounds. If true, the Anthomyiidae family could have developed olfactory receptor neurons that detect sulphuric compounds alongside the Scathophaga family which have their brooding grounds in dung (Ding et al., 2015). And the opposite could also be true, that *D. melanogaster* its relative species lost detection. More information is needed across phylogenetic and ecological divides to be able to pinpoint when and where the detection was gained alternatively lost in these species. Furthermore, could including more species into the olfactomic experiments help solve issues or at least give pointers to issues like the ones described in an article by (Mlynarek et al., 2020). In this study a team of canadian researchers looked at maggot damage on onion in four Canadian regions between *D. antiqua*, *D. platura* and *Delia florilega* (Zetterstedt) and found that they all cause damage to different extents depending on a multitude of conditions. But they also state that it is very hard to determine which species does the most damage due to them being very similar. There seems to be a lot of overlap and confusion in old and new literature regarding which Delia species has what as target species and maybe the overlap is wide and then olfactomics might be able to provide pointers as to what attracts these different species to the same wide array of host crops and in the future a Delia lure could maybe be developed.

The dataset in this thesis is too small to make a solid exploration and determine any evolutionary differences when just comparing two species and four different kinds of vegetables. However, one could theorise that developing datasets that have derived from data gathered by the use of olfactomics lead closer to the convergence and divergence between different species which stem from the same ancestral pool of insects. Putting this in context with this thesis, what could be for future research is to involve the mentioned species *D. radicum, P. rosae, S. flava* and others which are related to *D. antiqua* and *D. melanogaster* genetically or by ecology. This would give hints as to where and why convergence in ecology has occurred (or not) while still being diverse phylogenetically. This was the reasoning behind inclusion of *D. melanogaster* as the model species of flies; it could provide some useful information on convergence/divergence in ecology. Including *D. melanogaster* in the experiments showed that there were no responses to the sulphuric compounds which could be identified in yellow onion and napa cabbage which could indicate that either *D. antiqua* has developed receptors for these compounds or possibly that *D. melanogaster* has lost the ability to sense these sulphuric compounds.

4.3 Sustainability of IPM and the need for novel pest management tools

Pests around the world consume food that could feed approximately 1 billion people each year (E Birch et al., 2011) and at the same time the European Union's ban on neonicotinoids (Stokstad, 2018) make it seem more unthinkable to use pesticides such as neonicotinoids for managing pests. Integrated pest management (IPM) is an alternative to excess usage of pesticides and has long had a focus on economic and environmental aspects but this model needs to be expanded to include management, business and sustainability aspects and emphasise the importance of research and cooperation for the development of pest management (Dara, 2019). The 10 elements of agroecology contains the points of sustainability through co-creating and sharing of knowledge, efficiency of novel tools for increased sustainability and responsible governance which urges for collaboration through the whole chain from research to entrepreneurship to innovation to farmer and back again (Wezel et al., 2020). To align integrated pest management with the agroecological elements it is important to target specific pests without harming non-targets. Mapping the detection and preferences of many species could pinpoint the individual species' ecological niches and generating this comparative data could lead to novel lures and new tools for pest management. Data which has been generated through the large amounts of EAD papers written contain useful information in them and possibly many compounds which elicit responses in insects and attract them but lays dormant due to anonymity because of the vast number of papers. Olfactomics is an onset to deliver leads and could lead to more lures and leads if this data was collected in a comparable way. Because the truth is that even though advocates for IPM promote the sustainable forms of agriculture, reduced use in synthetic pesticides and solving the social issues that come with these, pesticide use has continued unabated (Deguine et al., 2021).

To translate these techniques of IPM which work by deriving results from research conducted through olfactomics into agroecology and the next step of IPM could be done to create solutions for sustainability in tandem with teaching and empowering farmers with knowledge. This transition is described by Deguine et al., 2021 as moving from IPM to Agroecological Crop Protection (ACP) and could be the next step in which IPM is further developed and intertwined with agroecology for a new paradigm shift in sustainable management of pests. ACP has three dimensions, it is a scientific discipline, strategy of cropping practices as well as a sociological movement within the socio-ecological landscape of food systems. There is large overlap between IPM and ACP but the idea is to move further beyond simple pest regulation by enhancing multiple ecosystem services through the mobilisation of biodiversity in ecosystems. This could be done at a basic level to utilise the results from olfactomics to counteract the natural cycles of pests, alleviating farmers while putting no non-target species or soil-health at jeopardy. Using olfactomics to identify a number of compounds which attract pests through aggregation, mating or feeding behaviour could be done through a number of techniques where the common denominator is the use of semiochemicals. These techniques which fall under the umbrella term of IPM and how they tie into olfactomics and semiochemicals will briefly be discussed to give an overview. Firstly is trapping and mass trapping as well as attract-and-kill. Trapping has been attempted in many settings ranging from agricultural land to orchards and from small areas from a few to thousands of hectares. The efficacy of trapping as well as attract and kill can vary a lot from study to study and even within studies. There are cases where the techniques work with high efficiency eradicating or near eradicating the populations (El-Sayed et al., 2006). However, there are also studies in which the techniques are applied year after year and there is no change in the population size (Navarro-Llopis et al., 2013). This indicates that mass trapping and attract-and-kill are techniques which depend a great deal on the spatio-temporal conditions of where they are deployed which in turn influences the success rate of the techniques. But the use of semiochemical and therefore olfactomics is essential in these techniques as well as the later ones and increasing the resolution and accuracy of attracting target species might drive on the efficacy.

Secondly is mating disruption which disorients one sex of a pest species, usually through sex pheromones, it is another method to disrupt the breeding potential of insect pests to diminish their reproductive capabilities and therefore populations (Chouinard et al., 2016; El-Sayed et al., 2006). Jallow et al., 2020 compared mating disruption to spraying of conventional pesticides in a setting of greenhouses in an attempt at controlling the South American tomato pinworm *Tuta absoluta* (Meyrick) and found that mating disruption in the right settings of 500 dispensers / ha-1 had an equivalent efficacy as the pesticide settings showing in potential mating disruption but also states in their article that more development is needed and field trials are crucial to work out the efficacy of the this technique. In another study, an attempt to control the Chestnut tortix moth *Cydia splendana* (Hübner) in Italy it was concluded that mating disruption attempts resulted in lower catchments of male individuals indicating that the technique had had an effect on the population and mating behaviour (Ferracini et al., 2021). In the case of mating disruption it could be interesting to move beyond pheromones and look towards semiochemicals for changing the behaviour and creating mating disruption through the application of olfactomics.

Sterile insect technique (SIT) is a technique that does not involve semiochemicals but which has proved successful in IPM. It involves releasing a high quantity of sterile individuals of a target pest species into an area where they can mate unsuccessfully with the other sex, preventing off-spring to be produced. This technique can lead to the eradication of a species population and is widely recognised as an effective technique in area-wide integrated pest management (AW-IPM) (Chouinard et al., 2016). Coupling SIT with some form of odour-based attractant might improve the efficacy of SIT, finding specific chemical compounds which could be laced onto the sterile insects could be one way to integrate olfactomics into the work of SIT.

The last technique for the application of olfactomics (covered in this thesis) is monitoring of insect pests which is useful in several settings when coupled with other techniques as well as standalone. Monitoring is useful for the identification, quantification and migration of insect pests in a system and to take actions if these need to be controlled (Prasad & Mathyam Prabhakar, 2012). Attractive volatiles could be applied to lure-traps, yellow sticky traps, pit-fall traps and others which then attract an insect species which is identified or counted or both. Then based on this information farmers are able to take action or abstain from it with a higher degree of certainty. Monitoring could therefore help in reducing applications of pesticides and pinpoint the instances during the year where application of pesticides are highly needed and pests present to repress or control instead of applying according to a schedule or a hunch. Moreover does this mean that if action is not needed then there will not be any unnecessary application of pesticides, reducing the applied amounts and therefore the agroecosystem has been less affected. Furthermore, monitoring can also be coupled with the other techniques mentioned above for these to be more efficient. By attracting only the target pest species counting is sped up and identification made redundant given that the attractant is highly specific.

Monitoring can be done manually by humans or using novel technologies like artificial intelligence for species identification and quantification (Cardim Ferreira Lima et al., 2020). The review mentioned provides examples on how lepidopteran species, fruit flies and borers can be monitored with fully automated systems which gets more accurate every time it is used. This takes less time than it would do for a human and provides accurate data for the farmers which they can then take with them in their spraying schedules. The weight lies on context and importance of knowledge for the farmers, what farmers do not know simply can not help them but monitoring could provide a bigger picture for the farmer as well as context (Bentley, 1989) and providing them with data on the presence or absence of pests could make a big difference on application patterns and management of insect pests.

4.4 Creating sustainability on the pillars of agroecology through fundamental research and knowledge sharing

This thesis gathered comparable data on *D. antiqua* and *D. melanogaster* across a set of pest-relevant headspace samples. The idea of generating comparative data is to make it readily available for others. Instead of the data being used in a single study, it could prove useful to other researchers to expand their knowledge in chemical ecology or other areas of research such as pest management. In this case the results are of considerable interest when developing lures for either *D. antiqua* or species that are related or share ecology. Comparative studies ties in with SDG 17 from the 2030 agenda of the UN (United Nations, 2015) but furthermore with the European commission press release about data sharing and how that will boost the innovation capacity in Europe (European Commission, 2012). Moreover, in addition to the European commission's press release from 2012 there was also an article published in 2015 which points at the incredible potential cooperation and data sharing could have in research development (Fecher et al., 2015). To create these efficient ways of working and sharing data it is essential to have ways of performing replication and have standardised workflows. The focus in this thesis has been on streamlining the processes of data collection, quality control, analysis as well as visualisation of data. This was performed using standardised data sheets that not only acted as an annotation platform but also integrated tools for rapidly calculating Kovàts RI in the workflow. As the data sheets are formatted in the same way, it allowed for a rapid translation into R where custom scripts allowed for both QC (quality control) and subsequent comparative analysis. A step that is missing in the process is the infrastructure of a stand-alone open access database. Such a database would also permit for standardising different workflows used by a variety of researchers making it possible for them to deposit their data into minable databases, making it possible to learn from each other saving large amounts of time and valuable research funding. In agroecology the three pillars of sustainability are the building blocks on which the science is founded (Gliessman, 2014). During the maste's programme there has been a lot of focus on sustainability, mostly on the social and economic sustainability of farmers and some on ecological sustainability, but the take on sustainability and agroecology in academia has not been very present. The 10 elements of agroecology include one named co-creating and sharing of knowledge which focuses on agricultural innovation for responding to challenges through cooperation (FAO, 2018). The ideal "production chain" in academia from an agroecological point of view flows from researcher to entrepreneurs where innovation integrates the needs of the end users, in this case the farmers. Communication and cooperation are keys in science, a skill that students in bachelor's and master's of agroecology are trained in. But science is still lagging behind in digitising and making information available for all. If researchers and teachers integrated open access to cooperative databases it would allow their students to develop an analytical mindset while working on large datasets. Individual researchers, especially the ones who have been doing research for a long time, have vast amounts of knowledge which tends to get lost if not published in a paper and even then it might get buried in the large amounts of papers being published. Furthermore, today researchers own their results and they are expected to themselves become incubators and entrepreneurs or their data is published and is many times lost just because so much is published each year. Making it hard for entrepreneurs and local farmers to acquire information from scientific literature. Databases of primary research data such as those gathered here would allow information to be disseminated throughout society. There are already open access databases which are ready to be integrated into the daily work of researchers. Some have been mentioned like LOTUS (Rutz et al., 2022) which is a database for sharing knowledge in natural products research, NPClassifier, Cactus, DoOR which is a database for the full Drosophila response profiling (Münch & Galizia, 2016), MZMine but also MACE which is a mass spectral database for chemical ecology (Schulz & Möllerke, 2022) giving free access to help researchers with their work in the elucidation of natural products in a GC-MS setting. In addition are there a plethora of open source coding packages, some which were used in this thesis, like chemodiv, ggplot, egg, seqRFLP and phylotools which could be powerful tools for integrating the data and making comparative studies. Because even though it is important to create databases for sharing of knowledge it is just as important to utilise these databases and integrate them into the workflow of research and interconnecting the different databases for novel forms of cooperation and comparative studies. This would then in turn hopefully lead to more efficient data generation and in turn innovation (Jiang, 2021; Karpen et al., 2021).

5. Further and future research

Further work on identifying compounds which attract specific pests is needed for the development of species specific-lures. In direct linking to this thesis species like *P. rosae*, *S. flava*, *D. platura*, *D. radicum* and other species with similar ecology or who are related genetically. It would be interesting to research these as they are also pests in temperate climates as *D. antiqua* is and are described in literature to have either similar or overlapping ecology or being close genetically. Moreover, would extensive field trials in different environmental settings provide useful data on how the flies are attracted to what in different environments or if the environment negates the efficacy of attracting the insects. Having an extended database on MS and EAD data on these pests as well as an extended set of host crops would lead to more specified lure and in turn traps which would disrupt the agroecosystems to a lesser degree compared to pesticides in today's conventional setting of spraying. Creating a database on this data would allow for other researchers as well as entrepreneurs and innovators to extract data and work on new solutions for an accelerated development of novel pest management tools for the sustainability of farmers and the environment.

Furthermore research on the equipment used in this kind of research would be most helpful. If the EAD equipment could be developed to be more efficient and have a higher throughput instead of recording times pushing the 1-hour mark from start to finish so much more could be done. The need for effective and dependent equipment is just as important for the mental health of researchers as well as for the transparency that work is being done correctly.

6. Conclusions

Mapping of volatilomes of yellow onion, napa cabbage, radish and carrot through GC-MS as well as identifying elicited responses of mentioned samples on insect species *D. antiqua* and *D. melanogaster* through GC-EAD is the core of this thesis. Data from GC-MS revealed different sets of volatile organic compounds in the crops as well as overlap in some compounds. Samples contained compounds from the different metabolic pathways: alkaloids, amino acids and peptides, fatty acids, polyketides, shikimates and phenylpropanoids and terpenoids as well as compounds which were not identified by NPClassifier. The interesting differences for the research of this thesis is the sulphuric compounds identified in yellow onion and napa cabbage. It could be concluded that *D. antiqua* elicit responses to some of these sulphuric compounds while *D. melanogaster* does not, indicating niche-driven evolutionary divergence at some point in time.

This study also highlights the usefulness and potential of open access and open information software and databases when utilised for research when researching non-model organisms where a lack of data is obvious. Databases like LOTUS, PubChem, NPClassifier and ClassyFire as well as programming packages in R have made it possible to conduct comparative research on the mentioned insect species as well as crops. By utilising these available tools by making further comparative studies there is a possibility to find lures to make novel pest management tools which give farmers alternatives to their current unsustainable pest management practices. Furthermore would a workflow utilising these databases alleviate the work of researchers so that researchers instead can focus on new discoveries instead of rediscovering already generated data which can be shared with other stakeholders like entrepreneurs and innovators who could apply the data and come up with solutions for the farmers' problems. These solutions would both increase the sustainability for farmers in terms of economic gains, reduce exposure to pesticides would boon their health but also increase the environmental sustainability and reduce the degradation that pesticides cause to the agroecosystems.

The data and the analytical workflow used in this study is openly available at [https://github.com/Aysaram/Masters_Thesis/releases/tag/v1.0].

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