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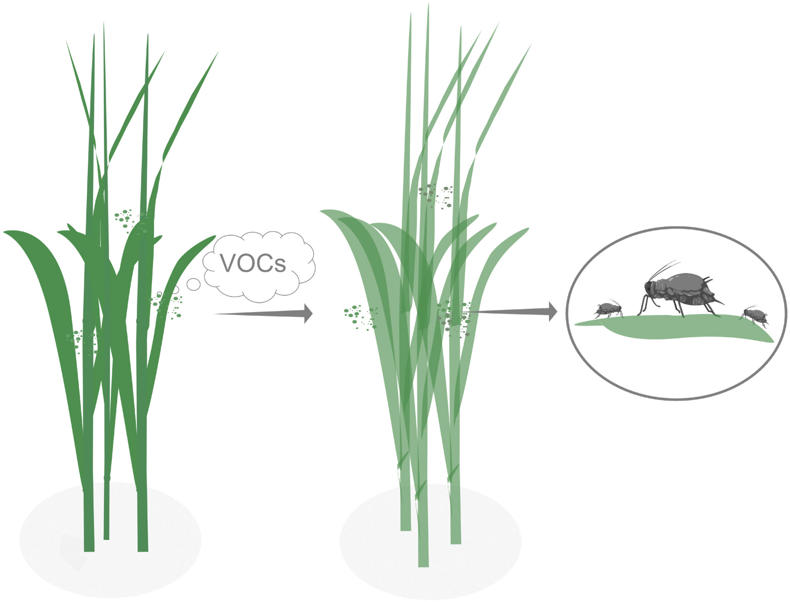
volatile plant communication as an underlying mechanism

Sokha Kheam



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volatile plant communication as an underlying mechanism

Sokha Kheam
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
Uppsala

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Cover: Volatile interactions between certain barley cultivars affecting aphids
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Swedish University of Agricultural Sciences, Department of Ecology, Uppsala, Sweden

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Insect-plant interactions within cultivar mixtures

Abstract

Promoting genotypic diversity of crops *via* cultivar mixtures presents a promising strategy to sustainably control insect pests and diseases, and thereby enhance yield. However, the underlying mechanisms responsible for reduced pest performance in cultivar mixtures remain unexplored. This thesis investigated the effects of volatile interactions between different cultivars in mixtures and their impact on insect pest performance. Two cultivars, initially growing at different rates, adjusted their growth to each other in the late stage due to aboveground chemical interactions. Volatile interactions between certain undamaged cultivars induced changes in volatile emissions of receiving plants, specifically the increased release of trans-β-ocimene and two unidentified compounds. The increases of trans-β-ocimene in receiving plants repelled aphids. Changes in receiving plants prolonged the non-nutrition uptake duration of aphid stylet penetration from epidermis to the phloem, but reduced the duration of active nutrition uptake in the phloem. It consequently decreased the aphid relative growth rate and intrinsic rate. A tropical field experiment showed that increasing soybean cultivar mixtures attract specific natural enemies at certain plant stages, while provide inconsistent effects on insect pests, with no changes in diseases or yield. The findings confirmed that volatile interactions between cultivars are context-specific, depending on the genetic identity of the emitter cultivar. This study sheds light on the significant role of volatiles in plant-plant interactions, affecting plant-insect interactions in cropping systems and increasing crop resilience against insect pests. This knowledge can help us develop integrated pest management by better understanding how complex mechanisms and processes in crop production systems can be and how we can influence them. It holds significant implications for both crop protection and the advancement of sustainable agricultural practices.

Keywords: variety mixtures, integrated pest management, plant-plant interactions, insect-plant interactions, genetic plant diversity, barley, soybean, aphid, predator

Insekt-växt interaktioner inom sortblandningar

Abstrakt

Att främja genetisk mångfald hos grödor genom odlingsblandningar ärar en lovande strategi för att kontrollera skadeinsekter och sjukdomar på ett hållbart sätt. Genom det ökas också avkastningen. Mekanismerna som leder till att problemen med skadedjur minskar i sortblandningar är dock ganska okända. I denna avhandling undersöktes hur flyktiga kemiska signaler påverkade interaktionen mellan olika sorter och deras påverkan på skadeinsekter. Två kornsorter, som inledningsvis växer med olika tillväxt, justerar sin tillväxt till varandra i det sena skedet på grund av kemiska interaktioner ovan jord. Exponering för kemiska signaler från vissa kornsorter ledde till förändrad avgivning av flyktiga substanser hos mottagande växter, särskilt en ökad frisättning av trans-β-ocimene och två oidentifierade ämnen. Den högre koncentrationen av trans-β-ocimen hade signifikant avskräckande effekt på bladlöss. I ett annat experiment visade det sig att förändringar hos mottagande växter också kan göra att bladlössen behöver längre tid för att penetrera epidermis och nå floemet med sugsnabeln. Samtidigt minskade tiden de ägnade åt aktivt näringsupptag i floemet. Det ledde till att både den relativa tillväxthastigheten och den inneboende tillväxthastigheten hos bladlössen blev signifikant lägre. Ett tropiskt fältexperiment visade att ökad blandning av sojabönssorter attraherar specifika naturliga fiender vid vissa växtstadier, samtidigt som de ger varierande effekter på skadedjur. Detta utan att förekomsten av sjukdomar eller storleken på skörden påverkades. Resultaten visar att flyktiga interaktioner mellan sorter är kontextspecifika. Den genetiska identiteten hos utsändarsorten påverkar genom att de sänder ut unika sammansättningar av flyktiga substanser. Denna kunskap kan hjälpa oss att utveckla integrerad skadedjursbekämpning genom att vi bättre förstår hur komplexa mekanismer och processer inom växtodlingssystem kan vara och hur vi kan påverka dem. I förlängningen kan vi få ett förbättrat växtskydd som främjar långsiktigt hållbara jordbrukssystem.

Nyckelord: kulturblandningar, integrerad skadedjursbekämpning, växt-växtinteraktioner, insekt-växtinteraktioner, genetisk växtmångfald, korn, sojaböna, bladlus, rovdjur

Dedication

To my beloved parents, who always support and give me wonderful opportunities to broaden my knowledge and skills through education. Though both of you did not happen to see this, you are on every page.

My sincere apologies, MOM, that I cannot physically say goodbye to you in 2021, due to my PhD mission and the COVID-19 pandemic restriction. I do hope this could make you proud of me.

The roots of education are bitter, but its fruit is sweet—Aristotle

Contents

List of publications

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

I. Gallinger, J., **Kheam, S.,** & Ninkovic, V. The role of plant-plant communication in growth development and competition*.* *(Manuscript)*

II. **Kheam, S.,** Gallinger, J., & Ninkovic, V. (2024). Communication between undamaged plants can elicit changes in volatile emissions from neighbouring plants, thereby altering their susceptibility to aphids. *Plant, Cell & Environment, 47*, 1543–1555. https://doi.org/10.1111/pce.14828

III. **Kheam, S.**, Markovic, D, Rubene, D., Ith, D. & Ninkovic, V. (2023). Volatile interactions between specific undamaged barley cultivar affect aphid feeding behaviour and performance. *Journal of Pest Science. 96.* 1049-1059. https://doi.org/10.1007/s10340-023-01592-x

IV. **Kheam, S.**, Rubene, D., Markovic, D., Ith, S., Uk, O., Soung, S., & Ninkovic, V. The effects of cultivar mixtures on the abundance of insect pests and natural enemies, diseases, and yield in tropical soybean cropping system. *(Biological Control – Under Review)*

The contribution of Sokha Kheam to the papers included in this thesis was as follows:

I. Co-author. JG designed the study with VN. Conducted the experiments and collected data with JG. JG Analysed data. JG wrote first draft manuscript. Developed the manuscript with other authors. Revised and edited the manuscript with authors for publication.

II. First author. Designed the study with VN and JG. Conducted the experiments and collected data with JG. Analysed data with JG. Wrote first draft manuscript. Revised and edited the manuscript with co-authors for publication.

III. First author. Designed the study with VN. Conducted the experiments and collected data with DM. Analysed data with DR and VN. Wrote the first draft of manuscript. Revised and edited the manuscript with co-authors for publication.

IV. First author. Designed experiment with VN. Conducted field experiment and collected data. Analysed data with DR and VN. Wrote first draft manuscript. Revised and edited with other co-authors for publication.

List of tables

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Abbreviations

EPG	Electrical Penetration Graph
VOCs	Volatile Organic Compounds
RGR	Relative Growth Rate
r_m	Intrinsic Rate of Increase
GC-MS	Gas Chromatography-Mass Spectrometry
HIPVs	Herbivore-Induced Plant Volatiles
FW	Fresh Weight
LA	Leaf Area
DW	Dry Weight
IPM	Integrated Pest Management

1. Introduction

Modern agriculture has resulted in notable advancements in productivity and efficiency, yet it has faced challenges such as environmental issues and requirement of adaptations for sustainable agricultural practices. Despite the increasing use of chemical pesticides, insect pests and diseases continue to cause significant losses in agricultural production, prompting rising concerns about their adverse effects on human health, the environment, and biodiversity (Manosathiyadevan *et al.* 2017). Pesticide use can promote the development insect pest resistance, and negatively affect beneficial organisms such as natural enemies, microbes, pollinators, and earthworms, as well as pollute water and air ecosystems (Khan *et al.* 2023). Furthermore, climate change currently poses a worldwide agricultural obstacle, potentially impacting the population dynamics and abundance of certain insect pests, by shortening the length of generations and increasing the development of new offspring for high-value specialty crops (Jha *et al.* 2024). It additionally promotes pesticide resistance of the most harmful insect pest species globally (Ma *et al.* 2021), hindering existing pest control efforts and leading to substantial economic losses.

Given the substantial ecological and evolutionary changes of insect pests in response to climate change and pesticides, the usage of pesticides must be optimized to improve resilience to insect pest pressure for sustainable crop protection. Hence, there is a need to develop alternative, sustainable, and efficient systems that can minimize the environmental impacts associated with modern agricultural practices (Tilman *et al.* 2002; Foley *et al.* 2011; Ma *et al.* 2021; Khan *et al.* 2023).

This chapter provides a brief overview of the effects of increased botanical diversity *via* volatile interactions between different cultivars in mixtures, potentially affecting insect pests and plant pathogens.

1.1 Botanical diversity for sustainable agriculture

Botanical diversity in crop systems can be a solution to reduce damage by insects, improve biological pest control, and increase food production (Ratnadass *et al.* 2012). An increase in botanical diversity at the landscape level can lead to suppression of insect pests *via* either top-down (affected by natural enemies), or bottom-up effects (affected by plants) (Gurr *et al.* 2003). Landscape complexity or botanical diversity can directly impact herbivore abundances by concentrating or diluting primary host crops in the cropping systems (Bianchi 2022). Increasing plant diversity at the local scale can encourage natural enemy abundance and improve biological pest control services in simple agroecosystems (Letourneau *et al.* 2011; Beaumelle *et al.* 2021). In particular, visual or odour masking within plant diversity can impact the landings of insect pests (Finch & Collier 2000). This, in turn, increases the movement of insect pests and their susceptibility to predators (Straub *et al.* 2014). Botanical diversity offers physical and chemical barriers, known as associational resistance, against insect pests (Barbosa *et al.* 2009; Malézieux *et al.* 2009; Dahlin & Ninkovic 2013).

These studies have demonstrated that botanical diversity plays an important role in suppressing insect pests and attracting natural enemies in cropping systems. Taken together, botanical diversity or increased plant diversity can regulate insect pests to some extent, in a context-dependent manner (Beaumelle *et al.* 2021). Therefore, the effects of botanical diversity can vary from landscape to field level, such as through crop mixtures or cultivar mixtures.

1.2 From crop mixtures to cultivar mixtures

Both crop and cultivar mixtures can be used to achieve beneficial effects on pest control, influencing crop yield (Lin 2011; Tooker & Frank 2012; Reiss & Drinkwater 2018).

Crop species mixtures, also known as intercrops, are characterized by the cultivation of two or more crop species within the same field (Vandermeer 1989). The incorporation of diversity in intercrops is designed to distinctly separate crop species in both time and space, promoting temporal and spatial niche differentiation and complementarity (Stomph *et al.* 2020). Intercrops offer benefits through more efficient utilization of resources (Jensen *et al.* 2020), reduced susceptibility to diseases and pests (Brooker *et al.* 2015), and the potential for increased and stable yield (Weih *et al.* 2021). Using different crop species for mixtures in cropping systems can be time-consuming and logistically challenging for the current agricultural production (Lin 2011; Tooker & Frank 2012). Cultivar mixtures are, therefore, more applicable for genotypic plant diversity practices in current cropping systems.

Cultivar mixtures vary in many characteristics, but have sufficient similarity to be grown together (Wolfe 1985). Recent studies have suggested that cultivar mixture is a promising strategy to suppress insect pests (Tooker & Frank 2012; Dahlin *et al.* 2018; Snyder *et al.* 2020), diseases (Mundt 2014; Vidal *et al.* 2020), and improve yield (Reiss & Drinkwater 2018). However, other studies have reported the lack of mixture effects on aphids (Mansion-Vaquié *et al.* 2019; Grettenberger & Tooker 2020). Plant–plant communication within specific cultivar mixtures can enhance crop resilience to insect pests (Shoffner & Tooker 2013; Grettenberger & Tooker 2016; Dahlin *et al.* 2018). Understanding the varied impacts of cultivar mixtures on insect pests, natural enemies, diseases, and yield, is crucial from both ecological and agronomic perspectives. This involves explaining the underlying mechanisms that contribute to these observed effects, which are currently poorly-understood. This knowledge is essential for the agronomic decision-making process, and the effective implementation of sustainable agricultural practices.

It has been speculated that volatile interactions within cultivar mixtures could be responsible for reduced aphid plant acceptance in the laboratory, and suppression of aphid population in the field (Dahlin *et al.* 2018). Several questions can therefore be posed:

- Do receiving plants respond differently in response to volatile interactions between different cultivar mixtures?
- Do volatile interactions between different cultivars affect aphid host plant search behaviour, feeding behaviour, and overall performance?
- Are there any mixture effects on the abundance of insect pests and natural enemies, diseases, and yield in the field?

1.3 Insect-plant interactions

1.3.1 Plant-plant interactions

Plants share complex habitats, where interactions with neighbouring plants and other organisms are inevitable. A diverse range of different volatile organic compounds (VOCs) is produced and released by plants, providing information about the plant's current physiological state (Dudareva *et al.* 2013). Such plant-emitted VOCs play important roles in the detection of neighbours, as well as inter- and intraspecific plant interactions, as they carry important information about neighbouring plants (Ninkovic *et al.* 2020). VOCs therefore play a key role as cues and signals in trophic interactions (Ninkovic *et al.* 2020), and can stimulate or prime defence responses in neighbouring plants (Brilli *et al.* 2019). Herbivore-induced plant volatiles (HIPVs) induce changes in the neighbouring plants which can lead to pest suppression (Karban *et al.* 2014), and can attract natural enemies (Dicke & Baldwin 2010). Volatile interactions between undamaged plants can also change the physiology of the receiving plants with subsequent influence on organisms at higher trophic levels (Ninkovic *et al.* 2006). This phenomenon, known as allelobiosis, could potentially be responsible for aphid suppression in cultivar mixtures. The VOCs perceived from neighbouring plants, therefore, can be essential for host plants to prepare for resource competition, self-protection against herbivores and microbes, or to attract natural enemies (Turlings & Erb 2018).

These studies provide evidence that plants receiving volatiles from neighbours can change their morphology and physiology to adapt growth and prepare for future competition or threat. This thesis extensively investigates how plants respond to specific neighbouring volatiles within cultivar mixtures, conducted in both laboratory and semi-field settings.

1.3.2 Insect responses to plant-plant interactions

The interdependence of insects and plants is evident in their evolutionary history. Insects rely on plant volatiles not only to identify the appropriate host plants, but also to assess the nutritional quality of the host and the presence or absence of other insects (Bruce & Pickett 2011). To achieve this, insects have developed intricate olfactory systems, primarily located on the antenna and maxillary palpi, consisting of various types of olfactory receptor neurons (Singh *et al.* 2019). The diverse olfactory receptor neurons in sensillae are specifically responsible for insect detection of plant volatiles (Bruce & Pickett 2011). Aphids, for instance, use sensory receptors to perceive characteristics such as colour, shape, texture, and odour emitted by plants (Bruce *et al.* 2005), and utilize the specific volatile blends or individual compounds to locate their host plants (Pettersson *et al.* 2017).

Aphid feeding behaviour and performance vary depending on host plant characteristics. Stylet penetration begins with short probes to the epidermal layer, followed by longer probes to the mesophyll tissues, and finally in phloem sap ingestion of the sieve element (Tjallingii & Esc 1993). For example, aphids spend twice as long in phloem-feeding, and have a longer duration of sustained feeding in susceptible genotypes, compared to resistant ones (Singh *et al.* 2020). The development time of *Rhoplaosiphum padi* was significantly delayed, and its intrinsic rate of increase was significantly reduced on the most resistant wheat cultivar, compared to susceptible cultivars (Jafari *et al.* 2020). Interestingly, exposure of one barley cultivar to volatiles from another cultivar reduced aphid acceptance in the laboratory setting, and reduced population growth in specific cultivar combinations in the field (Figure 1) (Dahlin *et al.* 2018). This provides a good indication that the observed reductions in aphid population could be due to the early delayed establishment of aphids in certain mixtures.

In order to understand these varied effects in cultivar mixtures on aphids, it requires the investigation of host plant search, feeding behaviour, and performance at the early stages of establishment. Therefore, this thesis aims to uncover an underlying mechanism *via* volatile interactions between cultivar mixtures, which could be responsible for reduced aphid feeding behaviour and performance in the laboratory settings.

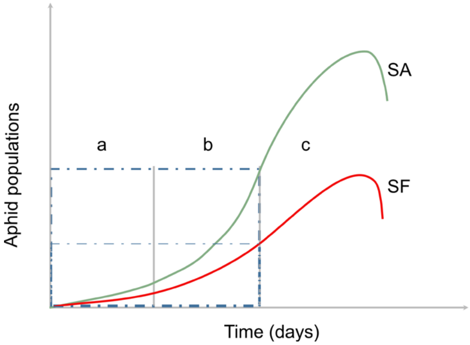


Figure 1: Field experiment examining barley cultivar mixtures (adapted from Dahlin *et al.* 2018). (a) early establishment of aphids, where winged or wingless aphids landed on the field, (b) represents the establishment of aphids in producing new offspring, and (c) represents the exponential increases of aphid population, which aphids rapidly continued to reproduce to the peak of the season. Green line represents Salome-Anakin (SA) mixtures, and red line represents Salome-Fairytale (SF) mixtures

1.3.3 Tritrophic interactions

In tritrophic interactions, bottom-up and top-down effects have been regarded as important ecological forces for enhancing integrated pest management (IPM) throughout the past 20 years. As bottom-up effect, certain barley cultivar mixtures (Salome-Fairytale) reduced the aphid population in the field (Dahlin *et al.* 2018), possibly attributed to the efficacy of volatile interactions between these cultivars. The reductions of insect pest populations can be also due to the resource dilution effect, causing difficulties in searching for host plant, resulting in less damage (Hambäck *et al.* 2014). As top-down ecological forces, diverse genotypic plant diversity has a positive impact on the abundance of natural enemies (Lin 2011; Han *et al.* 2022). Plant-plant interactions in certain cultivar mixtures can increase the resilience of crop fields to insect pests (Shoffner & Tooker 2013; Grettenberger & Tooker 2017), attract natural enemies (Ninkovic *et al.* 2011; Tooker & Frank 2012), and suppress diseases (Vidal *et al.* 2020). In contrast, there was reportedly no effect on aphid populations in certain cultivar mixtures of barley (Dahlin *et al.* 2018) and soybean (Grettenberger & Tooker 2020).

Cultivar mixtures indicated in field studies that insect pest populations can be reduced only in certain cultivar combinations (Dahlin *et al.* 2018). This thesis further expends a field-based study, aiming to closely examine the effects of soybean cultivar mixtures in a tropical cropping system.

2. Aim and structure

2.1 Aim of the thesis

The overarching aim of this thesis was to investigate the host search, feeding behaviour, and performance of insect pests in response to plant communication through volatile interactions between cultivars in mixtures, and examine the effects of cultivar mixtures in a tropical cropping system.

To attain this aim, the four specific objectives were to:

i. Explore the role of plant-plant communication between different barley cultivars on their growth and competition (Paper I)

ii. Investigate the effect of plant-plant communication on the volatile profiles of the host plant and its impact on aphid olfactory behaviour (Paper II)

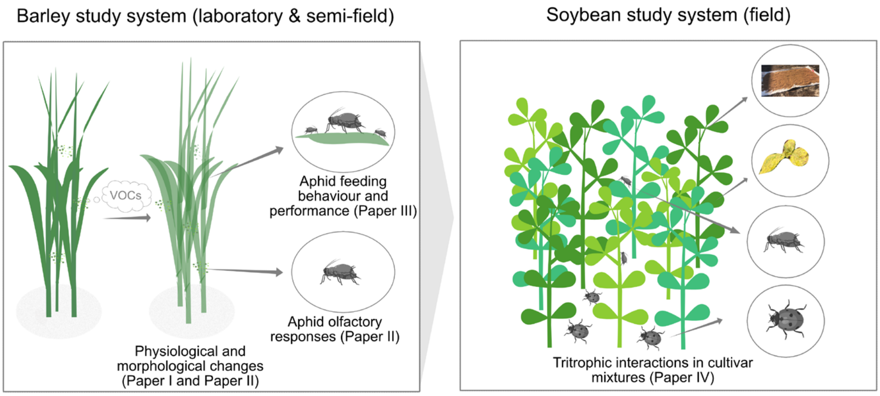
iii. Determine the impact of inducing plant responses due to volatile interactions between undamaged plants on insect feeding behaviour and performance (Paper III)

iv. Examine the effects of cultivar mixtures on the abundance of insect pests and natural enemies, diseases, and yield in a soybean tropical cropping system (Paper IV).

2.2 Structure of the thesis

This thesis originated from four papers, with its central focus on assessing the impact of volatile interactions in cultivar mixtures on insect behaviour and overall performance. The entire narrative of this thesis is illustrated in Figure 2.

Paper I, Paper II, and Paper III focused primarily on the barley cultivar mixture study system, while Paper IV extended the research to a new study system involving soybean cultivar mixtures under different climatic conditions (Figure 3). In the barley study system, Paper I mainly investigated the impact of volatiles on host plant growth adaptation and competition within barley cultivar mixtures. Papers II and III investigated aphid host-searching behaviour, feeding behaviour, and performance in barley cultivar mixtures. Building on the potential effects of cultivar mixtures observed in previous studies and the knowledge gained from the three barley papers, Paper IV extended the investigation to examine the effects of soybean cultivar mixtures on insect pest and natural enemy abundance, diseases, and yield in a tropical climate through a field experiment.



(Artwork by K. Sokha, 2024)

Figure 2: Illustrations of two different study systems for the entire thesis

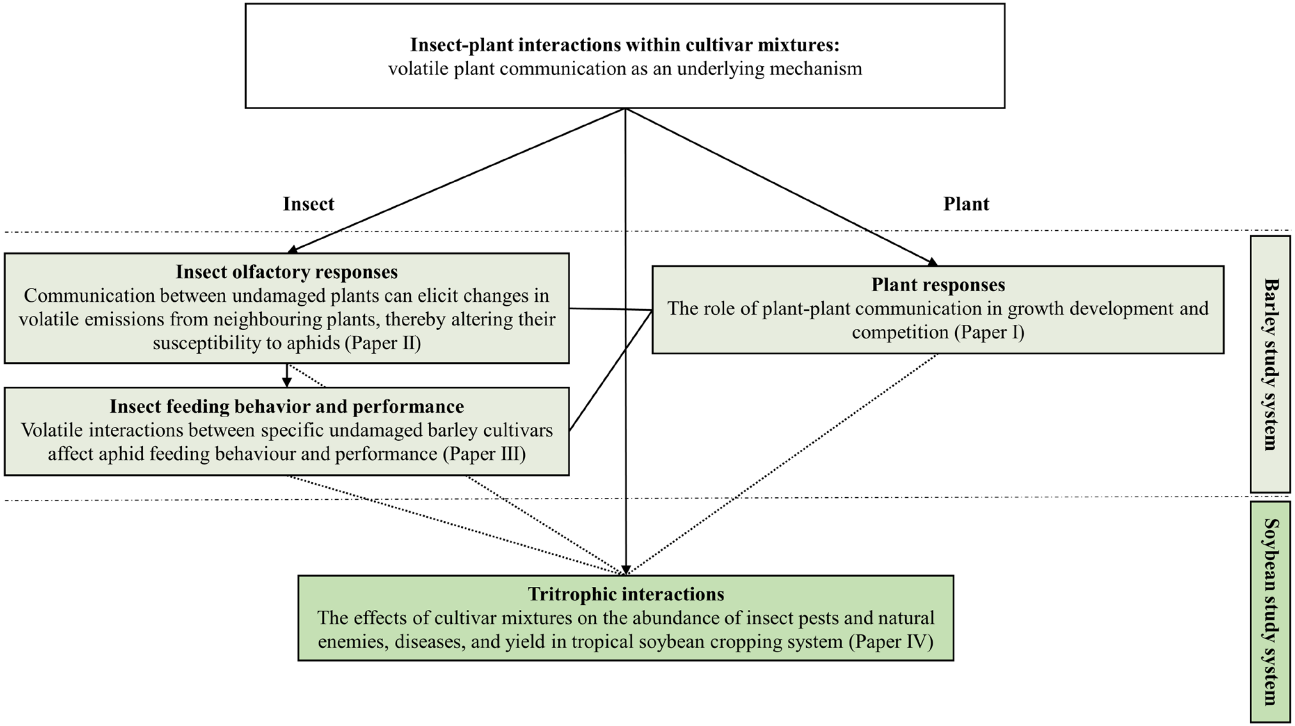


Figure 3: Thesis structure

3. Materials and Methods

In order to achieve the overall aim of this thesis, data were gathered from laboratory and semi-field experiments at SLU, Uppsala, Sweden, as well as from field experiments in Sweden, and another field experiment in Cambodia. The laboratory experiments were carried out in various seasons and years. Aphid feeding behaviour and performance were assessed in the spring and summer of 2020 and 2021 (Paper III), while host plant volatile emissions and aphid olfactory responses were assessed in the spring and summer of 2023 (Paper II). A semi-field experiment on barley plant growth and competition was conducted in the summer of 2021 at SLU's net yard (Paper I). The field experiment in Cambodia was conducted from June to November 2022 (Paper IV).

This chapter covers the detailed setup of model study systems, laboratory experiments, a semi-field experiment, and field experiments. It concludes with a brief summary of statistical analyses undertaken.

3.1 Model study systems

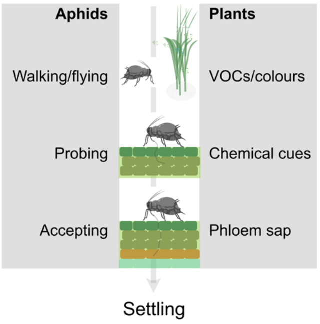
3.1.1 Insects – aphids as pests

Out of approximately 4,700 identified aphid species, around 250 are recognized as some of the most harmful insect pests for crops. Aphids belong to the extensive insect group in the superfamily Aphidoidea (order Hemiptera), primarily feeding on plant phloem (Lackman & Eastop 2000). Aphids damage crops by either directly feeding on phloem sap or indirectly transmitting viruses, resulting in reduced yield (Jarošová *et al.* 2016). Due to their rapid reproduction and the ability to spread, aphids emerge as significant pests, presenting considerable obstacles to both food security and the establishment of sustainable agricultural practices (Pettersson *et al.* 2017).

Aphids use visual and olfactory cues for their host-plant selection (Pettersson *et al.* 2017). Visual cues serve as the first stage in the host selection process for winged aphids and play a significant role in approaching and landing on a plant. Aphids respond to visual cues (colours) depending on their species and morphs. More importantly, aphids use olfactory cues from a wide range of semiochemicals associated with both host-plant discrimination and host-plant quality. Aphid responses to olfactory cues for host-plant recognition and their interactions are complicated.

Aphids use various chemical cues from plants for their host-plant orientation and settlement (Box 1). Firstly, aphids use plant volatiles as cues in finding their host plants. When aphids land on plants, they initially evaluate the surface of plants to prepare for their probing activity. Aphids initiate probing activity by responding to chemical cues within plant tissues (epidermis and mesophyll), using their stylet to puncture the plant tissues, primarily for assessing their host plants. Ultimately, aphids continuously utilize their stylet to access the phloem, where the quality of the phloem sap is crucial in determining acceptance or rejection of their host plants. If aphids experience thirst or dehydration, they might seek water from the xylem.

Box 1. Aphid-plant interaction process



The bird cherry-oat aphid (*Rhopalosiphum padi* L.) was used in bioassays (Paper II and Paper III). This species is one of the major pests in cereals, particularly wheat, oats, and barley, in temperate regions worldwide (Blackman & Eastop 2017).

Four groups of insect pests and natural enemies were concurrently surveyed within the soybean field experiment. The insect pest groups included whitefly, aphid, leaf beetle, and brown bean bug. The four groups of natural enemies consisted of predatory ant, lady beetle, dragonfly, and parasitoid wasp (Paper IV).

3.1.2 Plants – barley and soybean

Barley is an important cereal crop grown worldwide in temperate climates, serving as a food source for animals and humans. Several barley cultivars have been bred to increase resistance to the Russian wheat aphid (*Diuraphis noxia*), and greenbug (*Schizaphis graminum*) (Mornhinweg *et al.* 2012). However, a new barley cultivar that is resistant to *R. padi* has not yet been identified (Åhman & Bengtsson 2019).

According to a study (Dahlin *et al.* 2018), field investigations have demonstrated a significant reduction in aphid populations in mixtures of the barley cultivars Salome and Fairytale, compared to monocultures. However, this pattern was not present in the combinations when Salome was grown together with another cultivar, Anakin. Dahlin et al. (2018) found that volatile interactions caused a reduction in plant acceptance by aphids in Salome when exposed to Fairytale, but not when exposed to Anakin. Taking into account these findings, these three different spring barley cultivars Salome (Nordaat Saatzucht GmbH, Germany), Fairytale and Anakin (Sejet Plant Breeding, Denmark), were used in the present thesis (Paper I, Paper II, and Paper III). All three cultivars have different pedigrees: Auriga × (Publican × Beatrix) for Salome, Colston × (Receipt × Power) for Fairytale, and (Tumbler × Response) for Anakin. Scandinavian Seed AB, Linköping, Sweden, supplied seeds of the three cultivars.

Soybean (*Glycine max* L. Merrill) is a globally economically important crop, particularly in developing countries, due to its economic significance as a food source for both humans and animals. However, it suffers economic losses, mainly caused by insect pests (Musser *et al.* 2012), and diseases (Wrather *et al.* 2001). Only a few cultivars have been bred for moderate tolerance to bacterial pustule (*Xanthomonas axonopodis pv. glycine*) and brown bean bug (*Riptotortus* linearis) (Nget et al. 2022). Unfortunately, insects can quickly adapt to newly-developed resistant cultivars. This study used three different soybean cultivars: two commercial cultivars (98C81 and Santa Cruz) obtained from the Conservation Agriculture Service Center (CASC), the General Directorate of Agriculture (GDA) of Cambodia, and one traditional cultivar (Sbung) obtained from the local market. The cultivars were selected based on seed availability and their common use in local soybean production in Cambodia.

3.2 Laboratory experiments

3.2.1 Plant exposure

To thoroughly investigate the effects of volatile interactions between different barley cultivars on the receiving plants, twin Perspex cages (Ninkovic 2003) were used (Figure 4a).

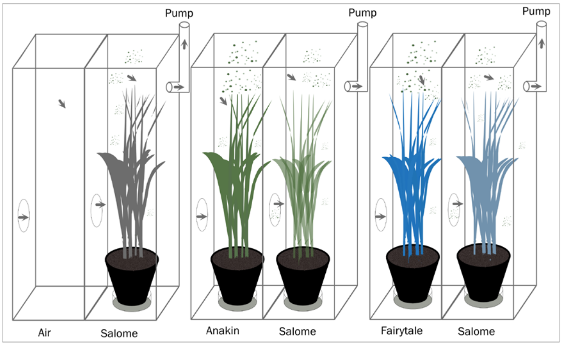
The cages were divided into two chambers: inducing and responding. The chambers (10 × 10 × 40 cm) were connected by a circular opening with a diameter of 7 cm in the middle wall. The air entered the system through the chamber containing an emitter plant, passed through the hole in the middle wall and exited thought the chamber containing a receiver plant before being vented outside the room. The airflow rate in the system was 1.2 L min^{-1}. To prevent potential interactions between plants *via* root exudates, each individual potted plant was placed in an individual Petri dish. Plants were watered daily for two minutes using an automated drop system (DGT Volmatic), without additional fertilisers. Before sowing, seeds were germinated between two filter papers in Petri dishes. The germinated seeds were sown per the pot (9×9×7cm) filled with P-soil (Hasselfors, Sweden) and kept in the growing chamber for nine days at 18–22 °C, 50–60% relative humidity, and L16:D8 h photoperiod. The plants were introduced into the exposure system at the one-leaf stage (seven days old), and exposed for five days under the same experimental conditions. The receiving cultivar, Salome, was exposed to Fairytale volatiles (SeF), Anakin volatiles (SeA), or air (Se0).

3.2.2 Plant volatile collections and analyses

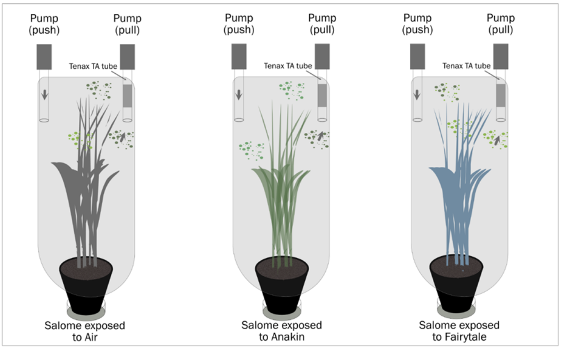
Headspace samples were collected from twelve plants per pot, including unexposed Salome, and Salome previously exposed to VOCs from Anakin or Fairytale, after a five-day exposure period (Figure 4b). The volatiles were collected using a push-pull system over 24 hours. Charcoal-filtered air was pushed into the oven bags at a rate of 600 mL min^{-1}, while simultaneously pumping air out of the bags over an adsorbent trap at 400 mL min^{-1}. The VOCs were collected using Tenax TA sample tubes (60/80 mesh size, GLScience, Eindhoven, Netherlands), containing 80 mg of adsorbent. To ensure quality control purposes, 1-nonene was used as an internal standard, with 20 ng injected on the top of the collection tube mesh immediately before headspace collections.

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the volatile emissions from receiving Salome plants (Figure 4c). The identification and quantification of volatile compounds were conducted using the "Automated Mass Spectral Deconvolution and Identification System" (AMDIS, V. 2.71; National Institute of Standards and Technology NIST, Boulder, CO) (Gross *et al.* 2019). Compounds were only reported as identified when their identities were confirmed through the analysis of authentic standards. Comprehensive details on plant volatile exposure, volatile collections, and analyses can be found in Paper II.

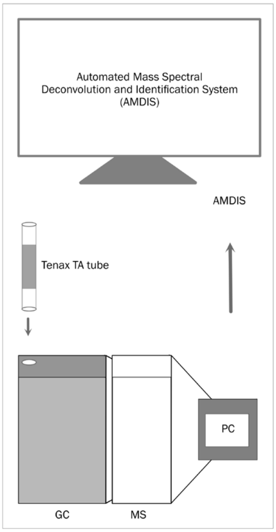
a). Plant exposure



b). VOCs collections



c). VOCs analyses



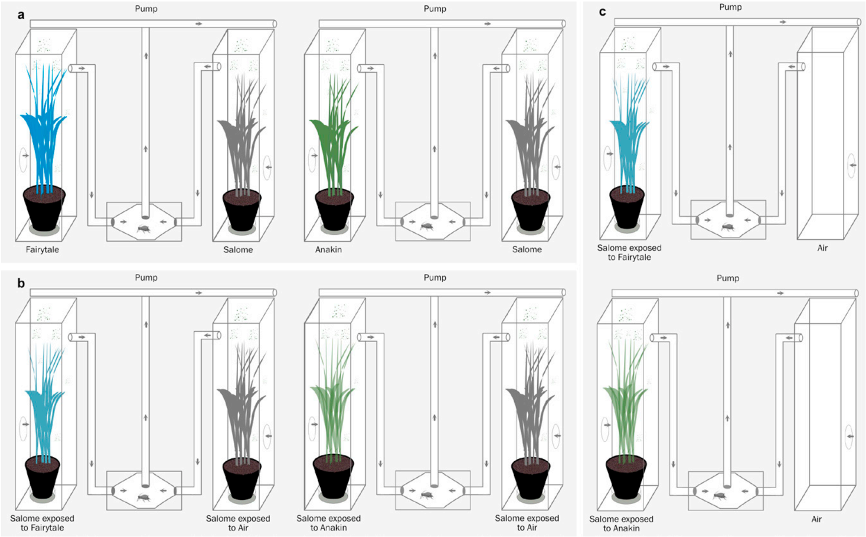
(Artwork by K. Sokha, 2024)

Figure 4: Illustrations of (a) plant volatile exposure, (b) volatile collections, and (c) volatile analysis protocols (adapted from Paper II)

3.2.3 Aphid olfactory responses to plants' odours

An olfactometer with two stimulus zones in opposite arms, and a neutral central zone, was used to measure the olfactory responses of aphids to volatiles released by the tested barley cultivars. The olfactory experiments (Figure 5) were conducted to compare aphid preferences and repellence for plant volatiles in the following combinations:

- *Experiment 1*: (a) Fairytale vs. Salome, and (b) Anakin vs. Salome,
- *Experiment 2:* (a) Salome exposed to Fairytale (SeF) vs. Salome exposed to clean air (Se0), and (b) Salome exposed to Anakin (SeA) vs. Salome exposed to clean air (Se0), and
- *Experiment 3:* (a) Salome exposed to Fairytale (SeF), and (b) Salome exposed to Anakin (SeA) vs. clean air from an empty cage (0).



(Artwork by K. Sokha, 2024)

Figure 5: Design of olfactory experiments. (a) olfactory experiment between emitter vs receiver cultivar, (b) olfactory experiments between exposed Salome to emitter(s) vs Salome exposed to air, and (c) olfactory experiments between Salome exposed to emitter(s) vs air (adapted from Paper II)

The experiments were conducted between 9:00 and 17:00 during the day, maintaining an average room temperature of approximately 20°C with a relative humidity ranging between 40% and 50%. One wingless aphid was placed into the central area of the olfactometer via a top hole. After a 10-minute adjustment period, the aphid's position in the olfactometer was recorded at 3-minute intervals for a total duration of 30 minutes. Each aphid underwent testing only once. Each replicate was defined by the total number of visits of a single aphid per single arm after ten recordings. Aphids that remained immobile for longer than 10 minutes were excluded from the analyses. Visits to the central zone were not considered in the analyses. The data were expressed as the total number of individual aphid visits per olfactometer arm during the observation period. More details of the plant volatile exposure and the olfactory tests can be found in Paper II.

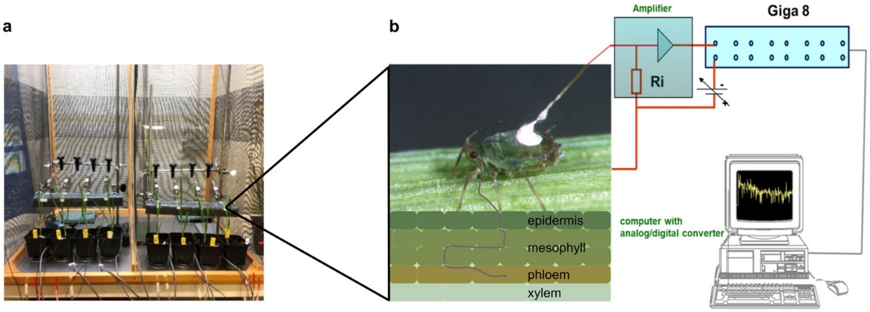
3.2.4 Aphid olfactory responses to an individual chemical compound (trans-beta-ocimene)

To confirm the olfactory responses of aphids to the increased volatile emissions of Salome exposed to Fairytale, an olfactory experiment was conducted on the dose olfactory responses of trans-β-ocimene. Serial dilutions were used based on the quantified amount in the headspace. Trans-β-ocimene (≥ 99% purity, Toronto Research Chemical Inc., Toronto, Canada), was diluted in methanol at five different concentrations (0.01, 0.1, 1, 10, 100 ng μl^{-1}). In this experiment, the olfactory responses of aphids to the various concentrations of trans-β-ocimene were compared to methanol as a control (Figure 6a, Paper II). Each diluted concentration of trans-β-ocimene, and methanol at a volume of 10 µg, was applied on small pieces of filter paper and allowed to evaporate for 1-2 minutes. This filter paper was then placed into glass tubes (2.5 mm diameter), connected to holes in the sides of the olfactometer arms. This method is described in greater detail in Paper II.

3.2.5 Aphid feeding behaviour and performance

Aphid stylet activities are commonly monitored by an Electrical Penetration Graph (EPG) device to determine the potential resistance occurring in different plant tissues (Tjallingii 2006). In this study, an eight-channel Giga-8 DC EPG system was used to investigate whether volatile interactions between barley cultivars affected aphid feeding behaviour through induction of plant resistance (Figure 6). The data were analysed using the “Stylet + a” software (EPG Systems) from the Stylet + d program (Tjallingii & Esc 1993). The software defines clear waveform patterns to determine the different phases of stylet performance during aphid penetration and feeding. The online EPG-Calc 6.1.7 software was used to calculate various EPG variables (Giordanengo 2014).

Twenty-nine different EPG variables were used to assess the aphid feeding behaviour on different treatments. Waveforms in certain phases of aphid feeding behaviour were selected for analyses, including none probing (NP_np), probing (Pr), probing pathway (C), potential drops (P_pd), sieve element salivation (E1), phloem sap ingestion (E2), stylet penetration difficulties (F), and xylem phase (G) (Tjallingii 1990). Primary analysis was conducted using Excel-VBA macro, as described by Giordanengo (2014). Statistical analysis was performed using 29 variables. The details of each phase and EPG setups were described in Paper III.



(Photo and Artwork by K. Sokha, 2024)

Figure 6: EPG experimental setup. (a) Eight channel EPG system, and (b) aphid feeding on barley leaves, attached to tracking monitor for aphid feeding behavior recording

Aphid performance was assessed by measuring their relative growth rate, intrinsic rate of increase, and development rate (Figure 7).

To evaluate aphid growth, 24-hour-old aphids were introduced to the receiving cultivar (Salome), after the plants had been exposed to volatiles from Fairytale, Anakin, or air, for five days. The observations took place in the exposing system, where receiving plants with aphids were continuously exposed to volatiles from emitting plants until the end of the experiment. The first instars (24-hour-old nymphs) were weighted using a microbalance

(Mettler Toledo, USA). One 24-hour-old nymph was placed on each receiving plant (Salome). After five days, each nymph was re-weighed, and the procedure was repeated. Aphid weights were used to calculate the mean relative growth rate, following the equation suggested by Radford (1967):

$$\text{MRGR } (\mu g/\mu g/\text{day}) = (\log W2 - \log W1) / t2 - t1$$

Where MRGR = mean relative growth rate, W1 = weight at the first weighting, W2 = weight at the second weighting, and t2–t1 = the time (days) between first (t1) and second (t2) weighting.

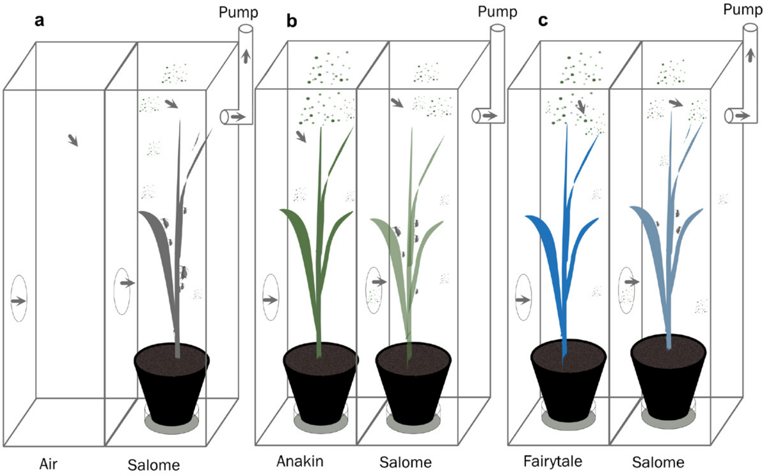
To observe aphid development time, first-instar (24-hour-old) nymphs of *R. padi* were placed between the first leaf and stem of a single Salome plant in the receiving cages. The nymphs were monitored until they produced their first offspring. The day of introducing 24-hour-old nymphs on the plant was counted as day 1. Aphid development time was calculated from this day to the production of the first offspring.

After observing development time, the intrinsic rate of increase (r_m) was recorded. Production of first offspring was recorded as day 1, and the total number of nymphs produced on each plant was counted over the following 5 days. The fecundity of an individual aphid to the intrinsic rate of increase (r_m) was calculated based on Wyatt and White (1977):

$$r_m = (\ln Md \times c)/d$$

Where Md is the number of nymphs produced by the adult in the first d days of reproduction after the adult moult. The constant (c = 0.738) is an approximation of the proportion of the total fecundity produced in the first days of reproduction.

Evaluation methods for aphid performance are described in more detail in Paper III.



(Artwork by K. Sokha, 2024)

Figure 7: Experimental design with twin cage system for the analysis of aphid performance on primed barley cultivars. (a) the exposure of Salome to air as control, (b) the exposure of Salome to Anakin, and (c) the exposure of Salome to Fairytale.

3.3 Semi-field experiment

Dahlin et al. (2018) showed that aphid population was reduced in barley cultivar mixtures (Salome-Fairytale) in the field, suggesting that these two cultivars may have interactive effects at the plant level. Therefore, a semi-field experiment was designed to investigate the impact of plant-plant interactions on their growth and competition.

To test this hypothesis, a semi-field experiment was conducted by sowing the two cultivars in plastic boxes (38 cm x 60 cm x 29 cm) filled with soil (Hasselfors P-soil, Sweden). The boxes were placed in a protected netted area at SLU Campus Ultuna in Uppsala, Sweden (Figure 8). Each box contained 100 seeds, arranged in four rows of 25 seeds per row. The planting configuration included a 2 cm distance within each row, 10 cm between the rows, and 4 cm from the edges of the box. Three treatments were analysed in the study: above- and belowground interactions, aboveground interactions, and single pure stands (no interactions) serving as the control. Measurements, including amount of chlorophyll, and biomass metrics such as fresh weight (FW), leaf area (LA), and dry weight (DW), were recorded. Additional information can be found in Paper I.

(Photos and artwork by G. Jannicke, SLU, 2022)

Figure 8: Experimental design of the semi-field experiment. (a) actual experimental design at net yard, SLU, Sweden, (b) two boxes of one treatment - only aboveground interactions, and (c) illustrations of three different treatments (above- and belowground interactions, only aboveground interactions, and monocultures), randomly arranged in the each block (adapted from Paper I).

3.4 Field experiments

3.4.1 Barley field experiments

Field experiments were conducted over two years, testing the mixtures of barley cultivars at one location (Uppsala) in 2020, and two locations (Uppsala and Norrköping), in Sweden in 2021. The experiments employed three barley cultivars (Salome, Fairytale, and Anakin), previously used in Paper I, Paper II, and Paper III. A randomized complete block design was arranged, consisting of six blocks, each containing six plots. The plot size was 60 m^2 (6 m x 10 m), with 1 meter distance of bare soil between plots.

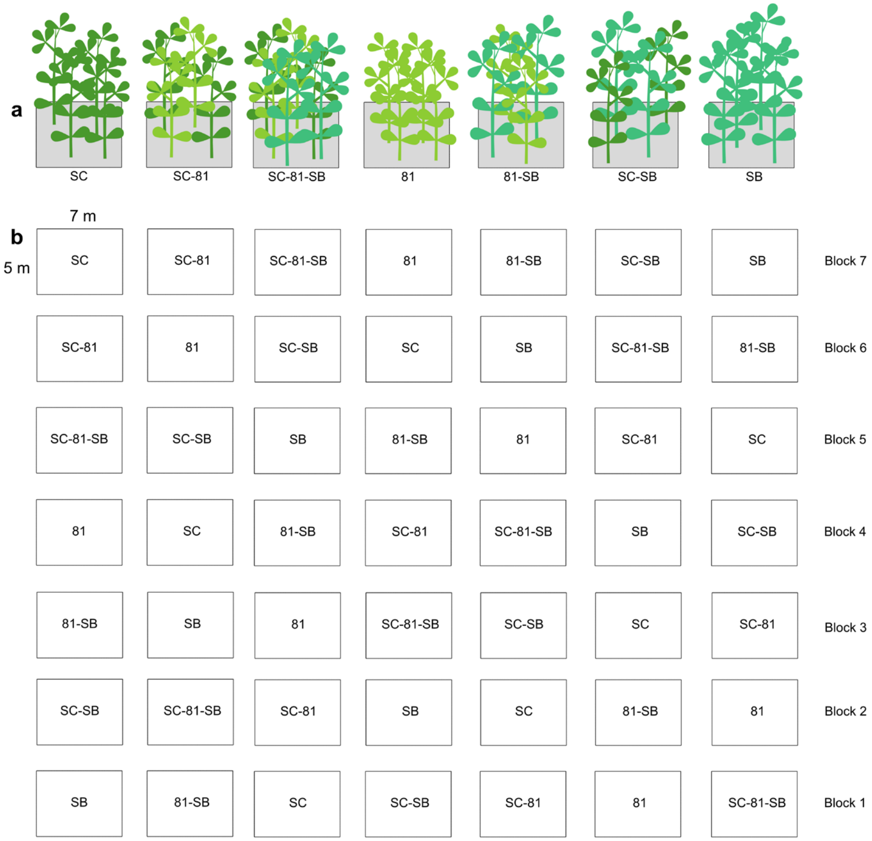
Aphids and lady beetles were monitored at weekly intervals. The number of aphids was counted on all plants in three randomly selected 1 m transects in each plot (Ninkovic *et al.* 2003). Simultaneously, two observers slowly walked along each long edge of the plot and counted the number of active adult lady beetles, observing as far as possible into their half of the plot

(Ninkovic *et al.* 2011). The number of lady beetles present in each plot was recorded in coordination as far as possible with the counting of the aphids.

3.4.2 Soybean field experiment

The study was expanded from the barley study system to include soybean in tropical climate conditions. This field experiment aimed to further investigate the effects of cultivar mixtures on the abundance of insect pests and natural enemies, diseases, and yield, in a soybean tropical cropping system, specifically in Cambodia.

The soybean field experiment was carried out from June to November 2022, covering an approximate area of 0.3 hectares. A randomized complete block design was used, consisting of seven blocks, each containing seven plots (Figure 9). Plot size was 35 m^2 (5 x 7 m), separated from others by a 1 m-wide stretch of bare soil. Two commercial cultivars (98C81 and Santa Cruz), and one traditional cultivar (Sbung) were tested. Soybean planting adhered to a specified configuration, and a seed rate of 55 kg ha^{-1} was applied for manual planting. Seeds were precisely prepared and mixed in equal proportions, with a mixing ratio of 1:1 or 1:1:1 for two and three cultivar mixtures (Table 1).



SC	SC-81	SC-81-SB	81	81-SB	SC-SB	SB	Block 7
SC-81	81	SC-SB	SC	SB	SC-81-SB	81-SB	Block 6
SC-81-SB	SC-SB	SB	81-SB	81	SC-81	SC	Block 5
81	SC	81-SB	SC-81	SC-81-SB	SB	SC-SB	Block 4
81-SB	SB	81	SC-81-SB	SC-SB	SC	SC-81	Block 3
SC-SB	SC-81-SB	SC-81	SB	SC	81-SB	81	Block 2
SB	81-SB	SC	SC-SB	SC-81	81	SC-81-SB	Block 1

(Artwork by K. Sokha, 2024)

Figure 9: Randomized complete block design of soybean field experiment. (a) Soybean plantation in block 7, where each colour represents different cultivars, and (b) Overall experimental design, arranged by 7 blocks, with 7 plots in each block. Each plot was 35 m^2 (5 x 7 m) with 1 m distance of bare soil between plots (adapted from Paper IV).

Various methods were employed to collect data on insects, diseases, and yield throughout the field experiment, to assess the potential impact of cultivar mixtures (Paper IV). Insect pest and natural enemy abundance, as well as disease occurrence, were comprehensively assessed across the entire experiment. Insect pest abundance, including aphid, whitefly, leaf beetle, and brown bean bug, was recorded on ten randomly selected plants per plot each week (Grettenberger & Tooker 2020). After insect pest counting, four groups of natural enemies (predatory ant, lady beetle, dragonfly, and parasitoid wasp), were also recorded. The counting was carried out by walking along the central line of each plot and recording the observed number of the natural enemies from both left and right side of the walking line for approximately five minutes per plot. Plant diseases were assessed by counting the number of infected plants based on observable symptoms on leaves at two reproductive stages (about 40 days and 54 days). Finally, yield was determined by harvesting three 1-meter transects in each plot at maturity in early November 2022. Throughout the field season, activities from soil preparation to harvest were conducted to ensure the feasibility of data collection (Figure 10). The detailed methodologies are outlined in Paper IV.

Table 1: Cultivars used in the study and the ratio of cultivar mixtures for the treatments.

No	Treatment	Code	Descriptions
1	Santa Cruz	SC	Pure stand
2	98C81	81	Pure stand
3	Sbung	SB	Pure stand
4	Santa Cruz - 98C81	SC-81	1:1 mixture of two cultivars
5	Santa Cruz - Sbung	SC-SB	1:1 mixture of two cultivars
6	98C81 - Sbung	81-SB	1:1 mixture of two cultivars
7	Santa Cruz - 98C81 - Sbung	SC-81-SB	1:1:1 mixture of three cultivars

(Photos by K. Sokha, T. Sophak, Cambodia, 2021)

Figure 10: Field activities from soybean experiment, Cambodia, 2021. Soil preparation (top left), vegetative growth stage, insect count, predatory ant hunting aphids, disease symptoms on leaves, and soybean seed dry (below right).

3.5 Statistical analyses

All data were analysed by using R statistical software (R Core Team 2022).

Paper I: Plant growth and competition

Generalized linear mixed models (GLMM) were used to determine the impact of interaction treatments (above- and belowground, aboveground and no interaction), and cultivars on growth parameters of barley plants. GLMMs were fitted to assess the effect of the interaction treatment, the cultivars, and the interaction of both factors, and blocking was incorporated as a random factor. GLMMs were fitted independently for every sampling time point with the glmmTMB function from the 'glmmTMB' package (Brooks *et al.* 2017). Type III analysis of variance was calculated with the ANOVA function from the 'car' package to determine the significant effects of factors and interactions (Fox & Weisberg 2019). Estimated marginal means (EMMs) and corresponding 95% confidence intervals were then calculated using the 'emmeans' package (Lenth 2022). These analyses are described in more detail in Paper I.

Paper II: Volatile organic compounds of profile compositions and aphid olfactory responses

To calculate and visualize differences of plant volatile compositions, the "vegan" package was employed (Oksanen *et al.* 2022). Non-metric multidimensional scaling (NMDS) plots were used to visualize Bray–Curtis dissimilarities of the VOC composition from Salome plants after exposure to air or volatiles from Anakin or Fairytale, calculated using the metaMDS function. Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test for discrimination between differently exposed plant volatile profiles. Fold change in the emission of single volatile compounds was calculated by the peak area per gram dry weight. In this case, the mean peak area per gram dry biomass from unexposed plants was calculated per day, accounting for daily variation. The logarithmic ratio of the peak area per dry weight from each exposed plant sample to the mean of unexposed plants from the corresponding day was calculated (Equation 1).

$$Fold - change = log\left(\frac{exposure_{day[i]}\left(\frac{peak\ area}{DW}\right)+1}{mean\left(control_{day[i]}\left(\frac{peak\ area}{DW}\right)+1\right)}\right)$$

To visualize the change of single compound emissions from Salome induced by exposure to volatiles from different cultivars, a heatmap was generated based on the mean fold-change. Generalized linear models with Gamma (log link) distributions were fitted to compare the fold-change between Anakin and Fairytale exposed Salome plants. The identity of the emitter cultivar and the sampling day were fitted as fixed effects. Differences in fold-change of single compounds from Salome plants exposed to Anakin or Fairytale were calculated using estimated marginal means and 95% confidence intervals, using the emmeans function from the "emmeans" package (Length, 2022). Additionally, the peak per g dry weight of trans-β-ocimene was analysed in the same way. Wilcoxon matched-pairs test was used to analyse the pair-choice test for aphid olfactory responses.

Paper III: Aphid feeding behaviour and performance

Due to the non-normal distributions of most EPG data for aphid feeding behaviour, the Wilcoxon rank-sum test (unpaired test) was used for the majority of the variables. For the variables that met the assumptions of parametric tests, general linear models (GLM) were employed (package lme4).

Generalized linear models (GLMER) were used to analyse response variables of aphid performance including development time, intrinsic rate of increase, and relative growth rate, by using the Gamma family with a log link. These statistical analyses are described in greater detail in Paper III.

Paper IV: Insect pest and natural enemy abundance, diseases and yield

Insect pest and natural enemy abundance data: Generalized linear mixed models (GLMER) were employed (package lme4), to analyse insect pest and natural enemy abundance (separate models for each taxon), disease occurrence, and yield. Due to the large variation in the number of aphids between plots, aphid data were scaled by using the mean value and standard deviation for analysis. The total number of natural enemies was also scaled when used as an explanatory factor in the models for insect pests. All models were validated by graphic examination of residual plots (Zuur *et al.* 2010). This methodology is more thoroughly detailed in Paper IV.

4. Results and discussion

This chapter presents the key findings of the thesis, providing a thorough discussion, and highlighting an alternative approach to sustainable agriculture and crop protection. The main focus is on in-depth exploration of how volatile interactions between different crop cultivars and insects, particularly aphids. This includes their search for host plant, feeding behaviour, and the success of their offspring. Aphids, much like other herbivorous insects, rely on plant volatiles as olfactory cues, which influence critical aspects of their behaviour and performance (Pettersson *et al.* 2017). The chapter examines how host plants respond to volatile interactions in specific cultivar mixtures, and how aphids respond to these interactions in laboratory settings. It finally concludes with a discussion of the soybean field experiment system.

The result of this thesis are summarized in Figure 11.

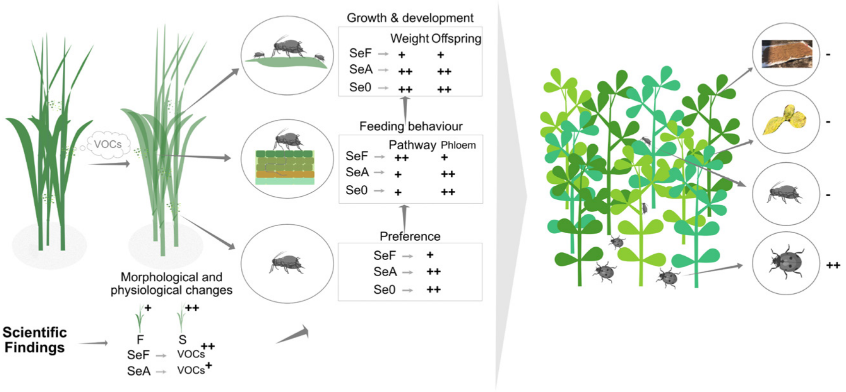
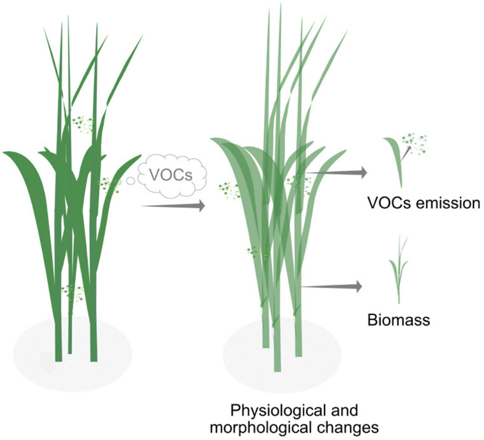


Figure 11: Summary of scientific findings presented in the thesis. (+) represents lower, (++) represents higher, and (-) represents no effect. Salome (S), Fairytale (F), Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to air (Se0).

4.1 Plant responses: growth, competition and changes in volatile emissions

Plants emit a variety of volatile organic compounds (VOCs), which provide information about their current physiological state (Dudareva *et al.* 2013). These VOCs are important for detecting neighbouring plants and mediating both inter- and intraspecific plant interactions (Ninkovic *et al.* 2016, 2020). In plant-plant interactions, VOCs act as significant cues, and can have the capacity to initiate or prepare defence responses in neighbouring plants (Heil & Karban 2010; Brilli *et al.* 2019). Paper I and Paper II in this thesis have specifically aimed to investigate plant responses to volatile interactions between cultivar mixtures (Figure 12).



(Artwork by K. Sokha, 2024)

Figure 12: Illustrations of volatiles affecting receiving plants

4.1.1 Plant responses to volatile interactions for growth and competition

Phenotypic plasticity, the ability to adapt to certain conditions, is crucial for plant fitness and successful establishment in a community. Plant adaptation to a competitive environment can alter the strength of competition and enable the co-existence of plants in a shared habitat (Hess et al. 2022). Aboveground interactions between plants are important in plant-plant communication, triggering their morphological and physiological changes in a shared habitat, thereby affecting their performance (Karban 2021). One element is in airborne volatile interactions between plants, where the exposure of certain cultivars to each other, could cause receiving plants to reallocate resources from aboveground to belowground growth (Ninkovic 2003). Specifically, Salome exhibited notably higher dry weight and relative growth rate compared to Fairytale when grown together or subjected to only aboveground interactions. However, both cultivars demonstrated similar dry weight and growth in monocultures at this particular time point (Figure 13a). In the later stage, the two cultivars had different growth rate in monocultures, with no differences in above- and belowground interactions and only above interactions (Figure 13b).

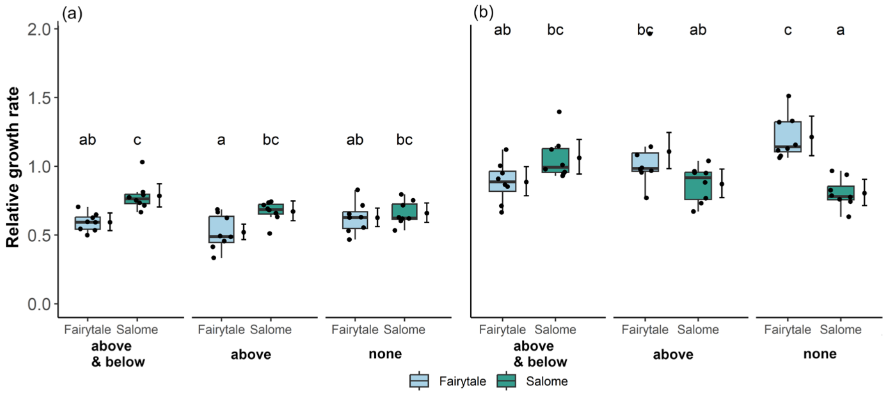


Figure 13: Comparisons of relative growth rates between three treatments: (a) 35 days and (b) 50 days. above & below: above- and belowground interactions between different cultivars, above: aboveground volatile interactions between different cultivars, none: pure stand/control without interactions between different cultivars. Letters above the bars represent statistical significance at $p \leq 0.05$ (adapted from Paper I).

Paper I reported significant differences in dry weight per plant and relative growth rate between Salome and Fairytale at early growth stage, which is also in line with Dahlin et al. (2020). However, the current study found that at the late growth stage, Fairytale and Salome fairly adjust their growth rates in response to both above- and belowground interactions and only aboveground interactions, with Fairytale outgrowing Salome in monocultures. These observations suggest that airborne interactions between different cultivars could be responsible for increased aboveground growth of plants at early stage, yet adjusting their growth at the later stage for a shared habitat with another cultivar. **Paper I highlights the adaptive changes in plant traits of one cultivar in response to volatiles from another cultivar,**

and confirms the role of airborne plant VOCs as important cues in plant-plant interactions and plant community dynamics (Effah *et al.* 2019). Early vigour is often viewed as a competitive advantage, as larger plants are able to acquire more resources (Andrew *et al.* 2015). The investment of resources is a trade-off between acquiring more resources and conserving and protecting (Herms & Mattson 1992; Pierce *et al.* 2017). Therefore, reducing investment in growth could allow reinvestment in other functions, such as defence mechanisms against pests. As a semi-field trial was conducted in natural conditions, the other aboveground factors, including light cues, mechanical signals, or wind direction could also have contributed to the observed effects. However, the arrangement of the boxes within each block was randomly distributed to minimize all these possible effects.

4.1.2 Volatile emissions of receiving plants after exposure

Given the observed effects between specific cultivars (Paper I), it was important to further explore the broader implications of plant-plant communication. Hence, Paper II investigated the volatile emissions of receiving plants affected by airborne communication between cultivars.

Plants can detect and respond to their environment through chemical cues, particularly the volatiles released by neighbouring plants (Ninkovic *et al.* 2016; Brosset & Blande 2022). Dahlin et al. (2018) found that the three barley cultivars, Salome, Anakin, and Fairytale, differed in volatile profiles. The analyses of fold change revealed a significant increase in the emission of trans-β-ocimene, and two unidentified compounds in receiving Salome when it was exposed to Fairytale, but no difference when it was exposed to Anakin (Figure 14). Specifically, the analyses of peak area per dry weight demonstrated a significant elevation of trans-β-ocimene in Salome exposed to Fairytale, compared to Salome exposed to Anakin or air (Figure 15). The current study therefore indicated that exposure to volatiles from certain cultivars induced changes in the volatile emission of receiving plants. The increases in trans-β-ocimene and two unidentified compounds suggest that Salome can detect and distinguish between volatiles released by different neighbouring cultivars. This could affect the physiological responses of neighbouring plants, possibly leading the activations of specific biological pathways to alter their own volatile emissions. A recent study has shown that belowground-damaged plants emit higher levels of trans-β-ocimene, enhancing resistance in neighbouring plants (Thompson *et al.* 2023). **Paper II reveals that exposure to volatiles from a specific undamaged cultivar can also enhance trans-β-ocimene production in undamaged plants. This is the first evidence that volatile interactions between specific undamaged plants can influence neighbouring plants to shift their physiological state, increasing the quantities of specific volatile compounds.** These findings therefore highlight the significant role of VOCs in interactions between undamaged plants.

Plants can distinguish between the volatiles emitted by different neighbours (Ninkovic *et al.* 2016, 2020; Kigathi *et al.* 2019). Volatile compounds emitted by undamaged onions were also found to enhance the release of specific volatiles from receiving potatoes (Ninkovic *et al.* 2013). **Altogether, evidence from Paper II indicates that plants can detect, differentiate and respond to, specific neighbouring plants, leading to distinct morphological and physiological changes.** Although the mechanisms of VOC perception in plants are not fully understood, the effects appear to be context-dependent (Hemachandran *et al.* 2017). The observed increase in specific volatile emissions in receiving plants may involve either an active (molecular and physiological changes inducing or priming defences), or passive mechanism (adherence of volatiles on the surface without further alterations) (Li & Blande 2015).

Based on the findings from Paper I and Paper II, **it is evident that volatile interactions between undamaged plants can affect the physiological and morphological state of receiving plants. These effects are mediated by alterations in plant metabolism, which may lead to an increase in the emission of specific volatile compounds and the growth adjustment in the receiving plants.** The findings demonstrate the complex ways in which plants interact and respond to each other, shedding light on the intricate interplay of chemical signalling in the context of plant communication. Given the observed potential impacts on receiving plants, it raises the question of whether insect responses could be altered as a result.

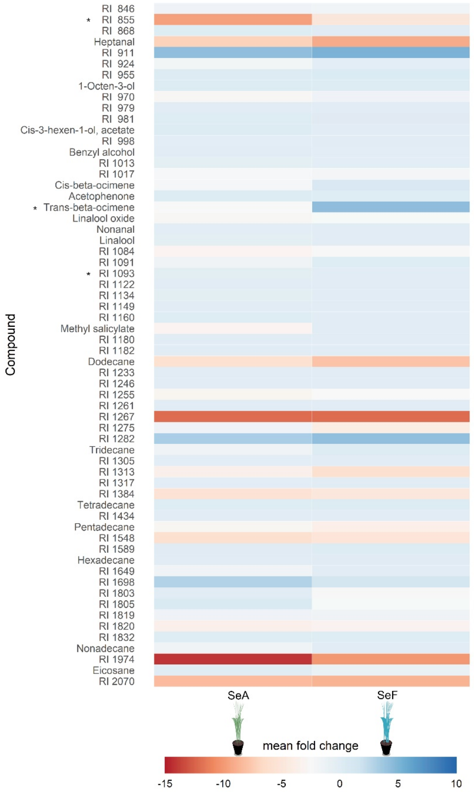


Figure 14: Fold change heatmap of VOCs between Salome exposed to Anakin (SeA) and Salome exposed to Fairytale (SeF). Asterisk indicates significant changes from GLM analyses ($p < 0.05$) (adapted from Paper II).

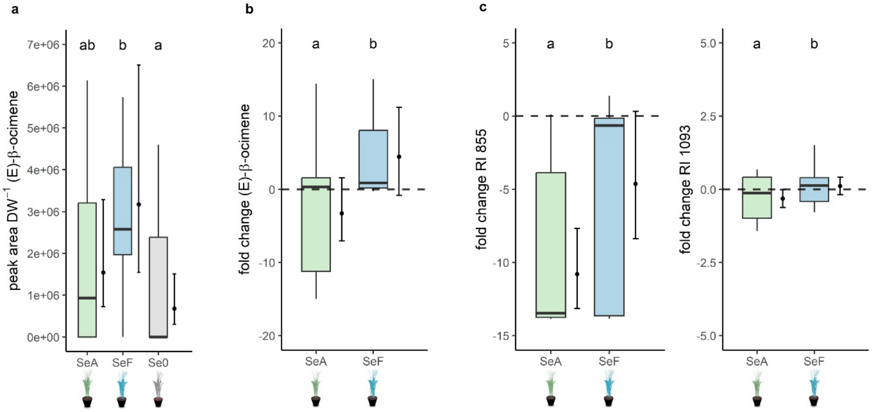
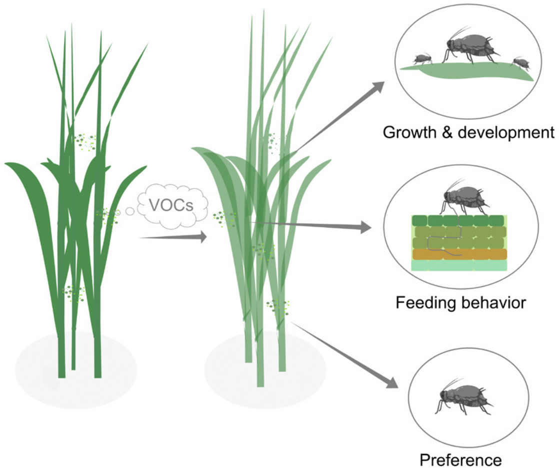


Figure 15 (see caption next page)

Figure 15: Comparisons in (a) the peak area per dry weight of trans-β-ocimene, (b) the fold-change concentrations of trans-β-ocimene, and (c) the fold change concentrations of RI 855 and RI 1093. Salome exposed to Anakin (SeA), Salome exposed to Fairytale (SeF), and Salome exposed to air (Se0). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level (adapted from Paper II).

4.2 Aphid responses to volatile interactions in cultivar mixtures: olfactory responses, feeding behaviour and performance

Aphids are known for their ability to detect and respond to plant volatiles, which can influence their decision to continue feeding or leave the host plants (as mentioned in section 3.1.1, Box 1). The current studies closely investigated aphid olfactory responses, feeding behaviour, and overall performance, which were influenced by volatile interactions between undamaged barley cultivars in mixtures (Figure 16). Therefore, the question that arises is: What are the effects on aphids when host plants modify their volatile emissions in specific cultivar mixtures?



(Artwork by K. Sokha, 2024)

Figure 16: Illustrations of volatile interactions between barley cultivars affected aphid preference, feeding behavior and performance.

4.2.1 Aphid olfactory responses

Plant volatiles play a crucial role in the detection and selection of host plants by insects, including aphids (Bruce *et al.* 2005). In accordance with the emitter-specific impacts on the VOC emission of receiving Salome, *R. padi* demonstrated emitter-dependent preferences for differently-exposed Salome plants. Aphids were less attracted to the odour of Salome when it was exposed to volatiles from Fairytale compared to when it was exposed to air. However, there was no preference shown for Salome that was previously exposed to Anakin or air (Figure 17). The current study showed that exposure to Fairytale causes a decrease in aphid attraction to Salome, indicating that changes in volatile emissions from specific emitters can affect behavioural responses of herbivorous insects. The reduced attraction and increased avoidance of aphids to the host plant volatiles may result from the effects of particular chemical compounds (Webster *et al.* 2010).

Trans-β-ocimene is a herbivore-induced plant volatile (HIPV) that effectively repel insect pests (Thompson *et al.* 2022). It induces defence mechanisms in recipient plants, leading to adverse effects on aphid olfactory responses, settling, feeding behaviour, and overall performance (Cascone *et al.* 2015; Kang *et al.* 2018). The observed higher concentration of trans-β-ocimene in Salome exposed to Fairytale volatiles triggered a significant avoidance response in aphids, suggesting its potential role in aphid host preference. This study highlights the potential role of trans-β-ocimene at certain concentrations in aphid repellence (Figure 5, Paper II). **Paper II supports the hypothesis that aphids can respond to specific compounds at particular concentrations, which can influence their search for a host plant** (Webster *et al.* 2010; Webster 2012). **Due to its repulsive effects on aphids, trans-β-ocimene may potentially be utilised for sustainable pest control in crop production.** That said, the ratios of each VOC may also affect aphid behaviour (Dardouri *et al.* 2019), and certain compound blends could be more repellent than individual VOCs (Bruce & Pickett 2011; Deletre *et al.* 2016).

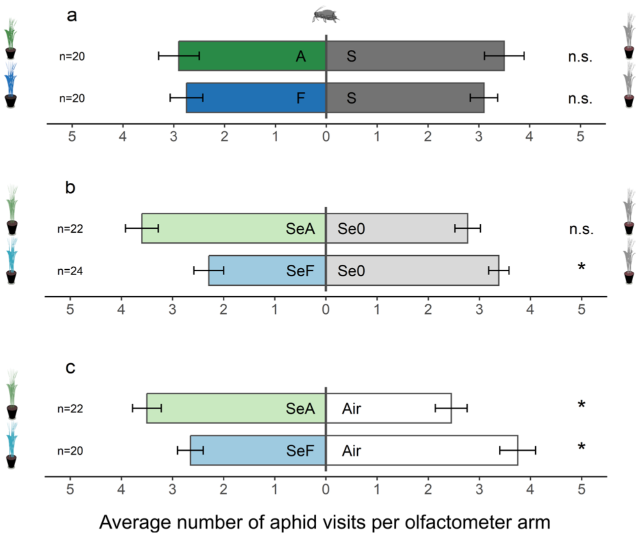


Figure 17: Aphid olfactory responses on plant odours. (a) emitter(s) *vs* receiving cultivar, (b) Salome exposed to emitter(s) *vs* Salome exposed to air, and (c) Salome exposed to emitter(s) *vs* air. Anakin (A), Fairytale (F), Salome (S), Salome exposed to Anakin (SeA), Salome exposed to Fairytale (SeF), and Salome exposed to air (Se0). Asterisk indicates significant differences according to the Wilcoxon signed-rank test ($p < 0.05$). n.s represents no significant differences between treatments (adapted from Paper II).

4.2.2 Aphid feeding behaviour and performance

Aphids, similar to numerous other herbivorous insects, rely on plant volatiles as olfactory cues or signals to make critical choices about their feeding behaviour, and overall performance (Pettersson *et al.* 2017). The results of EPG analyses showed that aphid feeding behaviour was disrupted in Salome when exposed to Fairytale, compared to Salome when exposed to air. No significant difference was observed between Salome exposed to Anakin and Salome exposed to air. Specifically, aphids in Salome exposed to Fairytale spent more time in the pathway phase, took longer to reach the first sustained phloem ingestion phase (t >1sE2), and had a shorter duration in the sustained feeding phase (s_sE2), than those feeding on Salome exposed to air. However, no significant differences were found between Salome exposed to Anakin, and Salome exposed to air (Table 1, Paper III). It is known that aphids must overcome host plant defences associated with the phloem in order to succeed in phloem sap ingestion (Tjallingii 2006). Phloem-based resistance may therefore induce changes in host plant morphology and physiology, which can reduce phloem sap ingestion as a result (Goodspeed *et al.* 2012; Simon *et al.* 2017). The prolonged duration that aphids spent in the pathway phase on Salome exposed to Fairytale indicates that volatiles from a specific undamaged plant could induce resistance factors in the epidermis and mesophyll of receiving Salome plants. The observed shorter duration of phloem ingestion further suggests the potential occurrence of phloem-based resistance in Salome exposed to Fairytale. However, it remains unknown whether volatile interactions between undamaged plants can induce epidermis, mesophyll, and phloem resistance factors in receiving plants. This may result in aphids struggling to engage in the phloem after salivation, or maintain phloem ingestion. It is likely from the findings of this thesis that volatile interactions between certain crop cultivars can upregulate certain defence genes and induce changes in phloem sap quality in the receiving plants.

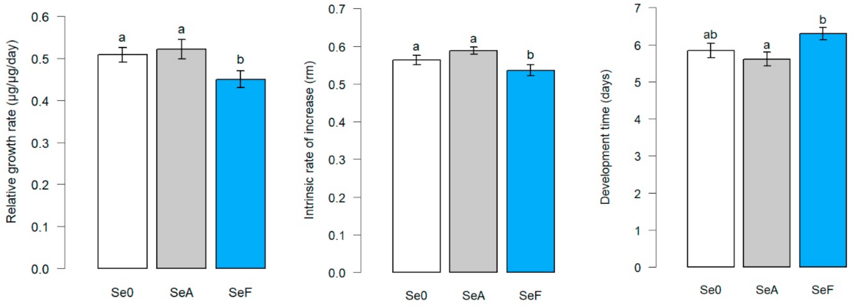


Figure 18: Aphid performance under different treatments: Salome exposed to air (Se0), Salome exposed to Anakin (SeA) and Salome exposed to Fairytale (SeF). Letters above the bars represent statistical significance at $p \leq 0.05$ (adapted from Paper III).

Aphid development time, fecundity, individual size, life span, and reproduction can be related to the quality of the host plant (Srisakrapikoop *et al.* 2021). The current study showed that aphid growth rate and intrinsic rate were significantly reduced in Salome after exposure to Fairytale, compared to Salome exposed to Anakin or air (Figure 18). The observed reduction in aphid growth corresponds to the aphid repellence and the disruption of feeding behaviour on Salome exposed to Fairytale, demonstrating a link between aphid olfactory response, feeding behaviour and performance. The observed reduction in aphid offspring and development on Salome exposed to Fairytale could be due to changes in the quality of phloem sap, as indicated by the shorter duration of phloem ingestion. The negative effects of volatile interactions on aphids are consistent with findings in companion plant studies (Dardouri *et al.* 2021; Ameline *et al.* 2023), and are similar to the reduction in *R. padi* performance observed in laboratory testing on wheat cultivar mixtures (Grettenberger & Tooker 2017).

In summary, Paper II shows that aphids exhibit a lack of preference for Salome exposed to Fairytale in their host-searching behaviour due to shifts in the volatile emissions of receiving plants. Paper III consistently establishes that volatile interactions in Salome exposed to Fairytale disrupt aphid feeding behaviour and performance, possibly due to the emergence of resistance factors from the epidermis to the phloem. Together, Paper II and Paper III provide compelling evidence that volatile interactions between certain undamaged plants can contribute to insect pest suppression, influencing aphid preferences, and disrupting aphid feeding behaviour and performance. These effects are attributed to induced changes in the physiology of receiving plants, highlighting the specificity of these interactions depending on the identity of the neighbouring plants.

4.3 Potential effects of volatile interactions in cultivar mixtures - from laboratory to field practice

4.3.1 Barley cultivar mixtures in field experiments – the challenges

Dahlin et al. (2018) revealed that Salome-Fairytale mixtures can reduce the aphid populations in the field when sown in separate rows. The results from the laboratory (Paper II and Paper III), and semi-field (Paper I) conditions in this thesis, provide concrete evidence that there are physiological effects resulting from volatile interactions between Salome and Fairytale. To provide a more practical perspective, field trials were carried out for 2 years (2020-2021) by using the three barley cultivars (Salome, Fairytale, and Anakin), and mixing in a 1:1 ratio. Unfortunately, aphid abundance was too low, which cannot be used for data analyses for these two years (2020 and 2021). The weather in 2021 was very dry (drought) (Figure 19), leading to the instability of barley growth and development in the field. Data from these field trials were not used in the analyses.

Apart from the lack of aphid field data, the hypothesis that volatile interactions between specific cultivars contributes to the reduction of aphid populations in Salome-Fairytale mixtures in the field (Dahlin *et al.* 2018), is entirely supported by the results presented in Paper I, Paper II, and Paper III. **These results conclusively affirm that volatile interactions between certain cultivars in mixtures, serving as an underlying mechanism, do indeed play an essential role in plant-plant communication, with implication for insect pest control**. This involvement has a robust impact on plant-insect interactions, specifically on the resilience of crops against insect pests. The findings contribute to our understanding of the complex interactions between plants and insects, and highlight their potential applications in pest management strategies.

(Photos by K. Sokha, Uppsala, 2021)

Figure 19: Barley field experiment affected by drought, Uppsala, Sweden, 2021. Reproductive stage (left) and harvesting time (right).

4.3.2 Soybean cultivar mixtures in tropical field practice

Although the data from the barley field experiments could not be used in this thesis, a new study system was introduced by using another crop (soybean) in tropical climate conditions, in Cambodia. Given the findings of Paper I, Paper II, Paper III, and previous studies on the potential of volatile interactions in cultivar mixtures, the outstanding question is how mixture effects may differ by climatic conditions. Therefore, a field-based experiment was used to determine whether the observed laboratory-based mixture effects could be applied to field practice in soybean cultivar mixtures.

Higher genetic diversity can increase the abundance of natural enemies in the field (Ninkovic *et al.* 2011; Tooker & Frank 2012; Grettenberger & Tooker 2017). The current study similarly showed the significantly higher abundance of lady beetles and dragonflies in the three-cultivar mixture (Figure 20). This suggests that natural enemies may be differentially attracted to higher genetic diversity of plants at a particular growth stage. This could be due to the greater diversity of microhabitats in three-cultivar mixture plots.

Natural enemies are known to locate their habitats and prey through detection of plant volatiles (Paré & Farag 2008). For instance, lady beetles respond to plant chemical cues. By extension, one crop cultivar exposed to another, or the combination of two different cultivars, are more attractive to lady beetles, compared to a single isolated cultivar (Ninkovic *et al.* 2011). The observed increased abundance of lady beetles and dragonflies in the three-cultivar mixture in this study could relate to the higher diversity of released plant volatiles. The results revealed that dragonfly and parasitoid wasp abundances were significantly higher in Santa Cruz-Sbung mixtures, compared to plots containing the individual cultivars (Figure 21). This suggests that Santa Cruz and Sbung could have a potential interactive effect in attracting natural enemies. It is therefore possible that plant-plant communication, *via* volatile interactions in cultivar mixtures, could contribute to the increased abundance of natural enemies, which would confirm bottom-up effects on natural enemy abundance. **Paper IV provides evidence that increasing genetic diversity through cultivar mixtures can have ecological effects in the field, increasing the abundance of certain groups of natural enemies at different plant growth stages. Effective selection of cultivars to be mixed could therefore optimize natural enemy attraction**.

Cultivar mixtures have previously been reported to reduce insect pest pressure (Pan & Qin 2014; Snyder *et al.* 2020; Nboyine *et al.* 2021). However, this study found inconsistent effects on insect pest abundance (Figure 1, Figure 2, Paper IV). The inconsistent effects on herbivore abundance were also observed in the context of soybean cultivar mixtures

(Grettenberger & Tooker 2020). Similarly, certain wheat mixtures (Mansion-Vaquié *et al.* 2019), and specific barley cultivar mixtures (Dahlin *et al.* 2018), were noted to have no mixture effects on aphid populations. The lack of a direct correlation between pest and natural enemy abundance in this study suggests a lack of top-down effect. This observation, along with the inconsistent effects of cultivar mixtures on insect pests, may be attributed to the relatively low insect pest populations in this particular study. Variability in agricultural landscapes impact herbivore abundance (Kheirodin *et al.* 2023). The low abundance of pests in this study may therefore be influenced by the diverse agricultural landscape in which it was undertaken, being surrounded by different crops such as cassava, bean, and maize. Changes in plant defensive responses, pest movement, or predator attraction at different population levels could potentially explain fluctuations in insect pest populations (Grettenberger & Tooker 2020). Alternatively, the observed inconsistent mixture effects on insect pests could be due to a lack of interactive effects between selected cultivars.

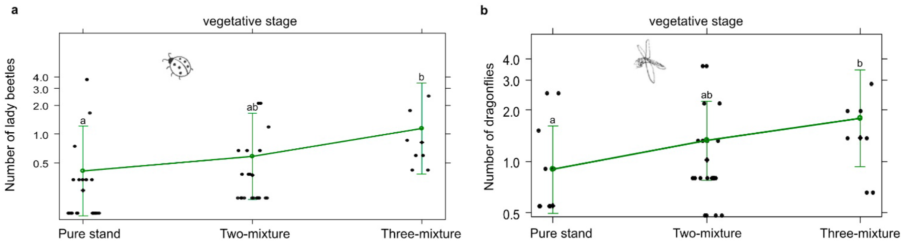


Figure 20: Comparisons in (a) the abundance of lady beetles, and (b) the abundance of dragonflies between pure stand, two-cultivar mixture, and three-cultivar mixture plots in the tropical soybean experiment. Letters above the bars represent statistical significance at $p \leq 0.05$ (adapted from Paper IV).

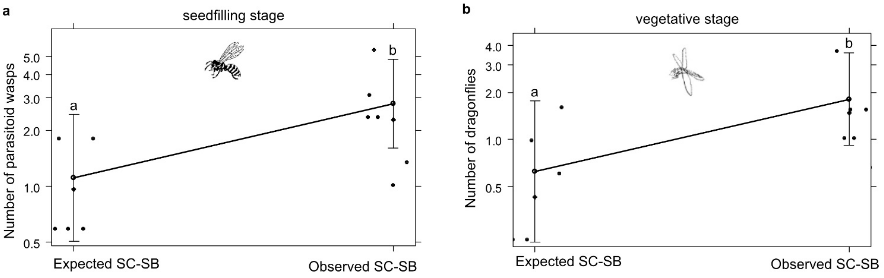


Figure 21: Comparisons in (a) the abundance of parasitoid wasps in seed filling stage, and (b) the abundance of dragonflies in vegetative stage between expected SC-SB vs observed SC-SB. SC-SB: Santa Cruz-Sbung. Letters above the bars represent statistical significance at $p \leq 0.05$ (adapted from Paper IV).

The diverse characteristics of individual cultivars have a significant impact on generating a broad spectrum of trade-off effects on insect pests, natural enemies, diseases, and yield. Although cultivar mixtures have been reported to reduce disease severity (Vidal *et al.* 2020), this study did not observe any mixture effects on the occurrence of disease symptoms on crop leaves (Figure 7, Paper IV). The lack of observed effects could be attributed to the disease evaluation method used in this study. Previous studies have suggested that cultivar mixtures can enhance yield production and stability (Tooker & Frank 2012; Reiss & Drinkwater 2018). However, the present study did not find any mixture effects on soybean yield (Figure 8a, Paper IV). The negative correlation between disease and yield (Figure 8b) highlights the importance of thoroughly assessing crop disease prevalence to understand the impact of cultivar mixtures.

Paper IV suggests that strategic selection of soybean cultivars for mixing can reveal their positive effects on biological pest and disease control. The study highlights significant differences between soybean cultivars in their effects on pests and natural enemies, susceptibility to diseases, and yield. This knowledge could be used to adapt cultivar selection to local conditions, particularly regarding specific pest and disease pressures.

5. Conclusions and future directions

This thesis has demonstrated that volatile interactions among certain cultivar mixtures are capable of suppressing insect pests. The final chapter provides a summary of the conclusions and future directions of the thesis.

5.1 Conclusions

In light of the findings and discussions presented in this thesis, the following specific conclusions can be drawn:

- Two cultivars, initially growing at different rates, adjust their growth to each other in the late stage due to aboveground chemical interactions, possibly *via* volatile interactions between different cultivars.
- Volatile interactions between specific undamaged plants trigger alterations in the volatile emissions of neighbouring plants, resulting in an increased emission of specific volatile compounds.
- The alterations in volatile emissions induced in recipient plants involve increased emission of specific compounds, such as trans-β-ocimene, which affects the behaviour of herbivores.
- Volatile interactions among certain undamaged plants can deter the searching behaviour of insects for host plants, disrupt their feeding behaviour, and affect their overall performance.
- Higher concentrations of trans-β-ocimene alter insect host preferences.
- Trans-β-ocimene, holds promise as a relevant component as a repellent for sustainable pest control in crop production.
- Increased genetic diversity through cultivar mixtures can attract specific groups of natural enemies in soybean fields.

- Cultivar mixtures in soybeans have inconsistent effects on insect pests, particularly under low pest pressure.
- Cultivar mixtures in soybeans do not influence disease prevalence or yield.
- The selection of cultivars with potentially interactive effects could serve as an effective approach to maximise biological pest control in sustainable soybean production.

Substantial evidence has indicated that insects respond differently to diverse ecological conditions in different environments. This thesis presents new findings by revealing the underlying mechanisms responsible for reduced aphid performance in barley cultivar mixtures. Specifically, it demonstrates that the deterrence and disruption of aphids depends on volatile interactions between particular cultivars, highlighting their dependence on the identity of neighbouring plants. This thesis also spotlights the necessity and the challenges involved in bridging the gap between controlled laboratory conditions and real-world agricultural settings. It provides valuable insights for the understanding of plant-plant communications, affecting plant-insect interactions in complex agricultural ecosystems. This thesis does also indicate that it could be difficult to control insect pests and diseases to enhance yield by using cultivar mixtures. By understanding the factors modulating variability in these interactions, further studies would provide new scientific evidence necessary for the development of cultivar mixtures as a feasible strategy for biological pest and disease control.

5.2 Future directions

Each individual paper has identified avenues for further investigation. The following future directions hold the potential to increase understanding of this mechanism from laboratory to field experiments for the advancement of sustainable agriculture and crop protection.

- The observed increases in emissions of specific volatiles in recipient plants could be attributed to either active or passive mechanisms of VOC perception. Further investigation is necessary to determine which mechanism is responsible.

- The two unidentified compounds which may act as cues for aphids in the detection and location of their host plants require further identification.
- Considering the repellent impact of trans-β-ocimene on aphids by specific exposed plants, deeper investigation of the tritrophic interactions involved is crucial. Therefore, future studies should focus on testing the effect of trans-β-ocimene on natural enemies, with a preference for lady beetles or parasitoids.
- The observed prolongation of the pathway phase, or the extended duration to reach sustained feeding may be due to potential resistance factors in the epidermis and mesophyll plant tissues. Therefore, it is essential to investigate gene expression in plants exposed to volatiles in effective cultivar mixtures to confirm that this can induce the up-regulation of genes in recipients.
- The observed disruptions in aphid feeding behaviour and performance in certain cultivar mixtures could be due to phloem sap quality; therefore, it is recommended that this should be examined further.
- Field experiments with barley cultivar mixtures did not provide conclusive evidence for the thesis due to the insufficient insect abundance and unfavourable climate conditions during the experiments. It is strongly recommended to conduct extended investigations through long-term field experiments involving the selected cultivars, preferably utilizing a 1:1 ratio in the mixtures to improve practicality and relevance for real-world agricultural practices.
- Inconsistency in mixture effects, and the absence of top-down impacts on insect pests within soybean cultivar mixtures, may be due to the low insect pest populations observed in the field trial. This emphasises the need for further investigations under higher insect pest pressure, and the identification of cultivars which respond effectively in mixtures.
- The impact of cultivar mixtures on diseases in soybeans requires further investigation. This can be achieved by using different cultivars in the mixtures, or evaluating various disease assessment methods.

- Given that the soybean cultivar mixture study was limited to a one-year field experiment at one location, it is difficult to assess whether the mixture effect persists over multiple years, locations, or cropping systems. For that reason, future studies should expand to include multiple years, diverse locations, or alternative cropping systems to explain the potential effects of cultivar mixtures.

This thesis provides clear evidence that volatile interactions between cultivars in mixtures, serving as a potential underlying mechanism in plant-plant communication, play a crucial role in pest management. Considering that insect pests can be suppressed by certain cultivar mixtures, the selection of cultivars which respond effectively in mixtures is highly recommended for pest control. This thesis acknowledges that the translation of effects observed in controlled laboratory conditions may pose challenges for field applications. Therefore, further studies need to investigate new methods for translating laboratory settings into field practice. The goal is to advance a comprehensive understanding of different aspects of this mechanism in cultivar mixture systems for sustainable crop protection, particularly in tropical cropping systems and beyond. This highlights the importance of bridging the gap between controlled environments and real-world field conditions to develop more practical and effective applications for sustainable agriculture practices. With substantial research evidence, cultivar mixtures can emerge as a reliable strategy for sustainable agricultural production.

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Popular science summary

Modern agriculture has achieved significant progress in enhancing productivity and efficiency, but has encountered obstacles including environmental concerns and the need to implement sustainable agriculture practices. Sustainable agricultural practices aim to meet the current and future needs for crop production while minimizing negative environmental, social, and economic impacts. An obstacle to achieving sustainable crop production is the efficient control of insect pests. Notably, sucking insects like aphids are considered significant pests in many agricultural systems, causing direct harm through feeding, and indirect damage by transmitting diseases. Presently, the typical approach to managing these pests involves the use of insecticides, which has adverse effects on both the environment and human health.

In the quest for more sustainable and effective strategies in agricultural production, increasing crop diversity through cultivar mixtures has potential for increased biological control against aphids and diseases. This thesis explores the complex world of plant-plant communication, investigating how interactions between different cultivars influence the behaviour and performance of insect pests, and how cultivar mixtures affect wider ecological interactions and diseases in tropical field conditions.

This thesis reveals that certain cultivar mixtures have the fascinating ability to suppress insect pests, showcasing a potential breakthrough in pest control. Two cultivars, initially growing at different rates, adjust their growth to each other in the late stage due to aboveground chemical interactions. The study shows that certain undamaged cultivar mixtures can induce changes in volatile emissions of neighbouring plants, leading to the increased production of specific compounds: trans-β-ocimene and two unidentified compounds. The increased releases of certain volatiles in receiving plants repel aphids. The higher concentrations of trans-β-ocimene can disrupt aphid preference. Given its repellence on aphids, trans-β-ocimene could potentially have a function in promoting sustainable pest control in crop production. Corresponding to the deterrence of aphids in certain cultivar mixtures, aphid feeding behaviour and performance are consequently disrupted. Specifically, the changes in receiving plants delay the non-nutrition uptake duration of aphid stylet penetration into the phloem, while reduce the duration of active nutrition uptake in the phloem. In other words, aphids struggle in engaging with, and feeding on plant tissues, thereby reducing their growth and development. These findings not only shed light on the complex dynamics of plant-plant communication but also highlight the potential for biological pest suppression. Expanding the scope of the study to tropical field practice, soybean cultivar mixtures can attract specific natural enemies at different plant growth stages. The impact on insect pests was inconsistent, with no effects on disease presence or yield. These findings suggest that effectively managing insect pests and diseases to improve yield through the use of cultivar mixtures may pose challenges for in-field application. Implementing cultivar mixtures as a viable strategy for biological pest and disease control may therefore require greater depth of scientific understanding.

In summary, this thesis indicates the capacity of plants to respond to chemicals produced by neighbouring plants by altering their growth and changing their own chemical emissions. It highlights the significant role of volatiles in plant-plant interactions, plant-insect interactions, and plant resilience against insect pests. It further suggests that different cultivars can complement each other differently in mixtures; the most complementary cultivars have potential to more effectively control pests and diseases. These findings have significant implications for crop protection and the advancement of sustainable agricultural practices.

Populärvetenskaplig sammanfattning

Det moderna jordbruket har gjort stora framsteg med att öka produktiviteten och effektiviteten. Men det finns utmaningar såsom miljöhänsyn och att jordbruksmetoderna ska vara långsiktigt hållbara. Hållbara jordbruksmetoder betyder att man möter nuvarande och framtida behov inom växtodling samtidigt som negativa miljömässiga, sociala och ekonomiska effekter minimeras. En av utmaningarna är att effektivt kunna kontrollera skadeinsekter. Sugande insekter, såsom bladlöss, anses vara särskilt betydande skadedjur i många jordbrukssystem. De orsakar både direkta skador genom sitt ätande på växterna och indirekta skador genom att de kan överföra sjukdomar. För närvarande är den vanliga åtgärden för att hantera dem att spruta insekticider, vilket har negativa effekter både på miljön och människors hälsa.

En strategi för mer hållbar jordbruksproduktion är att öka en grödas mångfald genom att blanda sorter. Det har visat sig ha potential för bättre biologisk kontroll av bladlöss och sjukdomar. Denna avhandling utforskar sådana blandningar. Det är en komplex värld av växt-växt kommunikation mellan de olika sorterna som, i nästa steg påverkar bladlössens beteenden och välmående. Avhandlingen har också studerat hur sortblandningar påverkar bredare ekologiska samspel och växtsjukdomar i tropiska förhållanden.

Resultaten visar visar att sortblandningar har förmåga att minska skadeinsekters angrepp, vilket visar på en potentiellt väg mot ett genombrott inom skadedjursbekämpning. Två kornsorter, som inledningsvis växte med olika hastighet, justerade efter att tag sin tillväxt till varandra på grund av kemiska interaktioner ovan jord. Dessa interaktioner ledde till att oskadade växter inom två sortblandningar kan påverka grannväxter att förändra sin avgivning av flyktiga ämnen. Det resulterade i ökad produktion av specifika föreningar: trans-β-ocimene och två oidentifierade föreningar. Detta hade en avskräckande effekt på bladlössen. I högre koncetrationer har trans-β-ocimene på egen hand avskräckande effekt. Det tycks därmed som trans-β-ocimene potentiellt skulle kunna vara en komponent för mer hållbar skadedjursbekämpning. I ett annat experiment visade det sig att förändringarna som den kemiska kommunikationen indicerat hos mottagande växter också kan göra att bladlössen behöver längre tid för att penetrera epidermis och nå floemet med sugsnabeln. Detta leder till att deras tillväxt minskade och utvecklingen gick långsammare. Dessa upptäckter visar både den komplexa dynamiken i växt-växt kommunikation och att det finns en stor potential för biologisk skadedjursbekämpning. Ett tropiskt fältexperiment visade att ökad blandning av sojabönssorter attraherar specifika naturliga fiender vid vissa växtstadier, samtidigt som de ger varierande effekter på skadedjur. Detta utan att förekomsten av sjukdomar eller storleken på skörden påverkades. Det visar på att det finns stora utmaningar för att nå en effektiv hantering av skadeinsekter och sjukdomar i fält. För att kunna utnyttja sortblandningar effektivt krävs en djupare vetenskaplig förståelse.

Sammanfattningsvis indikerar denna avhandling att växter har förmågan att reagera på flyktiga kemiska signaler som produceras av intilliggande växter genom att förändra sin egen tillväxt och justera sin egen avgivning av flyktiga ämnen. Den kemiska avgivningen ovanjord har en viktig roll både för hur växter interagerar med varandra och hur de påverkar de insekter som äter på dem. Det har därmed stor potentiell betydelse för växternas motståndskraft mot skadeinsekter. Vidare tyder det på att olika sorter kan komplettera varandra olika i blandningar; de mest kompletterande sorterna har potential att mer effektivt kontrollera skadedjur och sjukdomar. Kunskapen om detta har stor potential att kunna bidra till utvecklingen av nya och mer hållbara metoder inom växtskydd.

សង្ខេបវិទ្យាសាស្ត្រទូទៅ

កសិកម្មទំនើប សម្រេចបាននូវវឌ្ឍនភាពគួរឱ្យកត់សម្គាល់ក្នុងការបង្កើនផលិតភាពនិងប្រសិទ្ធភាព ខណៈពេលដែល ឧបសគ្គមួយចំនួនក៏ត្រូវបានជួបប្រទះរួមមានបញ្ហាបរិស្ថាន និងតម្រូវការក្នុងការអនុវត្តកសិកម្មប្រកបដោយនិរន្តរភាព។ ការអនុវត្តកសិកម្មប្រកបដោយនិរន្តរភាព មានគោលបំណងចម្បងដើម្បីបំពេញតម្រូវផលិតកម្មដំណាំនាពេលបច្ចុប្បន្ន និងអនាគត ដោយកាត់បន្ថយផលប៉ះពាល់អវិជ្ជមានលើបរិស្ថាន សង្គម និងសេដ្ឋកិច្ច។ ឧបសគ្គក្នុងការសម្រេចបាននូវផលិតកម្មដំណាំប្រកបដោយនិរន្តរភាពគឺ ការគ្រប់គ្រងប្រកបដោយប្រសិទ្ធភាពលើសត្វល្អិតចង្រៃ។ គួរកត់សម្គាល់ សត្វល្អិតជញ្ជក់ដំណាំ ដូចជា ចៃដំណាំ (aphid) ត្រូវបានគេចាត់ទុកថាជាសត្វល្អិតចង្រៃចម្បងមួយនៅក្នុងដំណាំកសិកម្មជាច្រើន ដែលបង្កផលប៉ះពាល់ដោយផ្ទាល់ តាមរយៈការស៊ីដំណាំជាចំណី និងការបំផ្លាញដោយប្រយោល តាមរយៈការចម្លងជំងឺដល់ដំណាំ។ នាពេលបច្ចុប្បន្ននេះ វិធីសាស្ត្រសំខាន់មួយក្នុងការគ្រប់គ្រងសត្វល្អិតចង្រៃ ពឹងផ្អែកយ៉ាងខ្លាំងលើការប្រើប្រាស់ថ្នាំសម្លាប់សត្វល្អិត ដែលមានឥទ្ធិពលមិនល្អដល់បរិស្ថាន និងសុខភាពមនុស្ស។

ក្នុងការស្វែងរកយុទ្ធសាស្ត្រប្រកបដោយនិរន្តរភាពនិងប្រសិទ្ធភាពបន្ថែមទៀត ក្នុងផលិតកម្មកសិកម្ម ការលើកកម្ពស់ភាពចម្រុះនៃហ្សែននៅក្នុងដំណាំ តាមរយៈការដាំដំណាំល្បាយពូជ ត្រូវបានចាត់ទុកថាជាជម្រើសជីវសាស្ត្រដ៏មានសក្តានុពលមួយប្រឆាំងនឹងសត្វល្អិតចង្រៃ និងជំងឺ។ និក្ខេបបទនេះ សិក្សាពីភាពស្មុគស្មាញនៃទំនាក់ទំនងរវាងរុក្ខជាតិ និងរុក្ខជាតិ ដោយសិក្សាស្វែងយល់ស៊ីជម្រៅពីទំនាក់ទំនងនៃសមាសធាតុគីមី (volatile) ដែលបញ្ចេញដោយរុក្ខជាតិរវាងពូជផ្សេងគ្នា លើអាប្បកិរិយាស៊ីចំណី និងការលូតលាស់របស់សត្វល្អិតចង្រៃ និងសិក្សាបន្ថែមពីឥទ្ធិពលនៃការដាំដំណាំល្បាយពូជ ទៅលើទំនាក់ទំនងអេកូឡូស៊ី និងជំងឺ ក្នុងលក្ខខណ្ឌទីវាលនៅតំបន់ត្រូពិច។

និក្ខេបបទនេះបង្ហាញថា ការដាំដំណាំល្បាយពូជមួយចំនួនមានសមត្ថភាពគួរឱ្យចាប់អារម្មណ៍ក្នុងការទប់ស្កាត់សត្វល្អិតចង្រៃ ដែលវាជារបកគំហើញថ្មីមួយដ៏មានសក្តានុពល ក្នុងការគ្រប់គ្រងសត្វល្អិតចង្រៃ។ ពូជពីរ ដែលមានកម្រិតលូតលាស់ដំបូងខុសគ្នា អាចសម្របការលូតលាស់របស់ពួកវាបាននៅដំណាក់កាលចុងក្រោយ ដោយសារទំនាក់នងគីមីផ្អែកលើដី។ ការសិក្សាស្រាវជ្រាវនេះ លាតត្រដាងឱ្យឃើញថា ការដាំដំណាំល្បាយពូជដែលមិនត្រូវបានបំផ្លាញមួយចំនួន អាចបណ្តាលឱ្យមានការផ្លាស់ប្តូរនៃការរំភាយសមាសភាពគីមីរបស់រុក្ខជាតិជិខាង តាមរយៈការបង្កើននៃការផលិតសមាសធាតុគីមីជាក់លាក់មួយចំនួនដូចជា៖ ត្រែនបែលតាអូស៊ីនីន (trans-β-ocimene) និងសមាធាតុពីរទៀតដែលមិនអាចកំណត់អត្តសញ្ញាណបាន។ ការកើនឡើងនៃការរំភាយសមាសធាតុគីមីរបស់រុក្ខជាតិជិតខាងទាំងនោះ អាចដេញចៃដំណាំបាន។

កំហាប់សមាធាតុគីមី ត្រែនបែលតាអូស៊ីនីនខ្ពស់ អាចរំខាន់ដល់ការស្វែងរកចំណីរបស់ចៃដំណាំ។ យោងតាមឥទ្ធិពលនៃការបណ្ដេញចៃដំណាំនៅក្នុងល្បាយដំណាំដាក់លាក់នេះ សមាធាតុគីមីត្រែនបែលតាអូស៊ីនីន អាចមានតួនាទីយ៉ាងសំខាន់ក្នុងការជួយបង្កើនការគ្រប់គ្រងសត្វល្អិតចង្រៃ ប្រកបដោយនិរន្តរភាពក្នុងផលិតកម្មដំណាំ។ ស្រដៀងគ្នាទៅនឹងឥទ្ធិពលនៃការបណ្ដេញចៃដំណាំនៅ ក្នុងល្បាយពូជដាក់លាក់នោះ អាកប្បកិរិយាស៊ីចំណី និងការលូតលាស់របស់ចៃដំណាំ ក៏មានការរំខាន់ផងដែរ។ ជាពិសេស ការប្រែប្រួលរបស់រុក្ខជាតិជិតខាង អាចពន្យាពេលយូរក្នុងការស្វែងរកចំណីមុខចូលទៅដល់ផ្លូវអែម (phloem) តាមរយៈការចាក់បញ្ចូលនៃម្ជុល (stylet) របស់ចៃដំណាំ ខណៈពេលដែលមានការកាត់បន្ថយពេលយ៉ាងច្រើនលើការជញ្ជក់សារធាតុចិញ្ចឹមជាចំណីសកម្មនៅក្នុងផ្លូវអែម។ ម្យ៉ាងវិញទៀតយើងអាចនិយាយបានថា ចៃដំណាំមានការលំបាកក្នុងការចាប់ផ្ដើម និងជញ្ជក់សារធាតុចិញ្ចឹមជាចំណីពីជាលិការុក្ខជាតិ ដែលបណ្ដាលឱ្យមានការកាត់បន្ថយនៃការលូតលាស់ និងការរីកធំធាត់របស់ពួកវា។ ការរកឃើញទាំងនេះ មិនត្រឹមតែបង្ហាញពន្លឺពីសក្ដានុពលដ៏ស្មុគស្មាញនៃទំនាក់ទំនងរវាងរុក្ខជាតិ និងរុក្ខជាតិប៉ុណ្ណោះទេ វាថែមទាំងបន្លិចចេញពីសក្ដានុពលក្នុងការបង្ក្រាបសត្វល្អិតចង្រៃបែបជីវសាស្ត្រផងដែរ។ តាម រយៈការពង្រីកវិសាលភាពនៃការសិក្សាទៅកាន់ការអនុវត្តទីវាលនៅតំបន់ត្រូពិច ការដាំសណ្ដែកសៀងល្បាយពូជ អាចរួមចំណែកក្នុងការទាក់ទាញសត្វល្អិតមានប្រយោជន៍មួយចំនួននៅដំណាក់កាលលូតលាស់របស់រុក្ខជាតិដាក់លាក់មួយ។ ឥទ្ធិពលនៃការដាំសណ្ដែកសៀងល្បាយពូជទៅលើសត្វល្អិតចង្រៃ មិនមានភាពស៊ីសង្វាក់គ្នានោះទេ ហើយវាក៏មិនមានការប្រែប្រួលលើជំងឺដំណាំ ឬទិន្នផលឡើយ។ លទ្ធផលទាំងនេះផ្ដល់ជាយោបល់ថា ការគ្រប់គ្រងសត្វល្អិតចង្រៃ និងជំងឺលើដំណាំប្រកបដោយប្រសិទ្ធភាព ដើម្បីបង្កើន ទិន្នផលតាមរយៈការប្រើប្រាស់ដំណាំល្បាយពូជ អាចប្រឈមបញ្ហាជាច្រើនក្នុងការអនុវត្តនៅទីវាល។ ដូច្នេះ ការអនុវត្តនៃការដាំដំណាំល្បាយពូជ ដែលអាចចាត់ទុកជាយុទ្ធសាស្ត្រលើការគ្រប់គ្រងសត្វល្អិតចង្រៃ និងជំងឺដំណាំ ទាមទារឱ្យមានការស្វែងយល់យ៉ាងស៊ីជម្រៅខាងផ្នែកវិទ្យាសាស្ត្រ។

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II

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ORIGINAL ARTICLE

Communication between undamaged plants can elicit changes in volatile emissions from neighbouring plants, thereby altering their susceptibility to aphids

Sokha Kheam[1,2] | **Jannicke Gallinger**[1] | **Velemir Ninkovic**[1]

[1]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2]Department of Biology, Faculty of Science, Royal University of Phnom Penh, Phnom Penh, Cambodia

Correspondence
Velemir Ninkovic and Sokha Kheam, Department of Ecology, Swedish University of Agricultural Sciences, Sweden.
Email: velemir.ninkovic@slu.se and sokha.kheam@slu.se

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Abstract

Plant volatiles play an important role in intra- and interspecific plant communication, inducing direct and indirect defenses against insect pests. However, it remains unknown whether volatile interactions between undamaged cultivars alter host plant volatile emissions and their perception by insect pests. Here, we tested the effects of exposure of a spring barley, *Hordeum vulgare* L., cultivar, Salome, to volatiles from other cultivars: Fairytale and Anakin. We found that exposing Salome to Fairytale induced a significantly higher emission of *trans*-β-ocimene and two unidentified compounds compared when exposed to Anakin. Aphids were repelled at a higher concentration of *trans*-β-ocimene. Salome exposure to Fairytale had significant repulsive effects on aphid olfactory preference, yet not when Salome was exposed to Anakin. We demonstrate that volatile interactions between specific undamaged plants can induce changes in volatile emission by receiver plants enhancing certain compounds, which can disrupt aphid olfactory preferences. Our results highlight the significant roles of volatiles in plant–plant interactions, affecting plant–insect interactions in suppressing insect pests. This has important implications for crop protection and sustainable agriculture.

KEYWORDS

aphid, aphid host plant orientation, barley, insect–plant interaction, plant–plant communication, plant–plant interaction, volatile organic compounds, volatile profiles

1 | INTRODUCTION

Plants share complex habitats, where interactions with neighbouring plants and other organisms are inevitable. They also use diverse signals and cues to interact with their neighbouring plants including: responses to the quality of light (Keuskamp et al., 2010), acoustic signals (Appel & Cocroft, 2014), root-exudates (Biedrzycki et al., 2010), root emitted volatile organic compounds (VOCs) (Delory et al., 2016), mechanical touch (Markovic et al., 2014) and airborne VOCs (Ninkovic et al., 2013). A diverse range of different VOCs are produced and released by plants, providing information about the plant's current physiological state (Dudareva et al., 2013). Such plant-emitted VOCs serve important roles in the detection of neighbours, as well as inter- and intraspecific plant interaction mediators, as they carry important information about neighbouring plants (Ninkovic et al., 2016, 2020). The VOCs perceived from neighbouring plants, therefore, can be essential for host plants to prepare for resource competition, self-protection against herbivores and microbes, or natural enemy attractions (Turlings & Erb, 2018).

Damaged as well as undamaged plants have been shown to release distinct volatile compounds that can affect tritrophic interactions in the ecosystem (Erb et al., 2015; Ninkovic et al., 2013). Plant VOCs play vital roles as cues and signals in plant–plant interactions (Ninkovic et al., 2020) and can stimulate or prime defense responses in neighbouring plants (Brilli et al., 2019; Heil & Karban, 2010). When plants are attacked by herbivores, herbivore-induced plant volatiles (HIPVs) are released (Clavijo McCormick et al., 2012), which function as direct repellents for herbivores or as attractants for natural enemies (Aartsma et al., 2017; Turlings & Erb, 2018). Furthermore, plants use volatile cues from undamaged plants to gather information on their neighbours and adapt to competition with proper morphological and physiological responses (Kegge & Pierik, 2010). This phenomenon, so-called allelobiosis, could possibly account for insect suppression in mixed plantings (Pettersson et al., 2003). Volatiles from onion plants can induce changes in potato volatile emissions making them less attractive to aphids in the laboratory and field experiments (Ninkovic et al., 2013). Previous studies further suggest a high specificity of such volatile-based plant–plant interactions depending on the species or even cultivar identity of interacting plants (Karban et al., 2006; Kheam et al., 2023; Ninkovic et al., 2013). For example, volatiles emitted from one specific barley cultivar significantly affect biomass allocation in another cultivar, leading to increased root biomass production, while other cultivars do not (Ninkovic, 2003). A growing body of literature provides substantial evidence that volatiles from undamaged plants can shift a variety of properties in neighbouring plants; however, it is still not well documented whether volatile interactions between undamaged plants from different cultivars affect plant volatile emissions and subsequent interactions in insect herbivores.

The evolutionary history of insects and plants is highly interconnected in nature. Insects use plant volatiles as cues to assess not only whether they are selecting the right host plant species but also to determine the nutritional quality of the host and the presence/absence of other insects (Bruce & Pickett, 2011). For instance, the damaged *Rumex confertus* released high levels of VOCs, that potentially repelled the weevil *Hypera rumicis* L. at high concentrations of 25 and 125 ng mn^{-1} (Piesik et al., 2015). Another weevil (*Sitophilus granaries*) was also repelled by high concentrations (100 and 1000 ng mn^{-1}) of most cereal volatiles (Piesik & Wenda-Piesik, 2015). The filbert aphid, *Myzocallis coryli* Goetze, showed low acceptance of leaves of certain cultivars of hazel (*Corylus* L.) with a high content of phenolic acids (Gantner et al., 2019). Phloem-feeding insects can use olfactory cues to identify suitable host plants, despite the evaluation of food quality, determined by the abundance of primary and secondary metabolites in the plant sap (Gallinger & Gross, 2020; Gallinger et al., 2020). Therefore, insects have evolved sensitive olfactory systems, located mainly on the antenna and maxillary palpi that consist of different types of olfactory receptor neurons has evolved in insects (Singh et al., 2019), to precisely recognize chemical cues in the surrounding environments (Field et al., 2000). The different olfactory receptor neurons with unique molecular structures in sensillae are responsible for insect volatile detections (Bruce & Pickett, 2011). In particular, aphids can utilize the sensory receptors to detect colour, shape, texture and odour released by plants (Bruce et al., 2005) and they can use specific volatile blends or single compounds to locate their host plants (Pettersson et al., 2017). These studies indicate that certain concentrations of specific plant volatiles could affect different groups of insect host-searching behaviours.

Aphids with their rapid growth and potential to spread, are major pests that pose substantial challenges to food security, and the development of sustainable agricultural practices (Pettersson et al., 2017). Chemical insecticides are still extensively used in cropping systems and have resulted in evolutionary responses in aphids. Aphids have led to the development of individual resistance (Foster et al., 2017) and their defense against natural enemies through the acquisition of heritable symbionts (Oliver et al., 2003). Therefore, the rapid development of alternative strategies to control aphid populations is needed for sustainable crop protection. One of those potential biological alternatives against aphids could be based on the effects of plant–plant communication via volatile interactions in genotypically diverse cultivar mixtures. Previous studies reported that plant–plant communication in certain cultivar mixtures can increase the resilience of the crop to insect pests (Dahlin et al., 2018; Grettenberger & Tooker, 2016; Kheam et al., 2023; Shoffner & Tooker, 2013). Field studies showed that significant decreases in aphid populations were observed in the mixtures of Salome and Fairytale barley cultivars as compared to their pure stands. There was no such pattern in the Salome and Anakin mixtures compared to pure stands. Laboratory studies showed that the volatile interactions reduced plant acceptance by aphids in Salome exposed to Fairytale (SeF), but not in Salome exposed to Anakin (SeA) (Dahlin et al., 2018). Additionally, aphid feeding behaviour was disrupted and performance was reduced in SeF, but not in SeA (Kheam et al., 2023). The results of these studies suggest that plant–plant volatile interactions between specific cultivars may contribute to changes in host plants that make them less suitable for aphids. However, the mechanisms of volatile interactions in specific cultivar mixtures against aphid host plant searching behaviours has yet to be established. A better understanding of the effects of volatile interactions in cultivar mixtures on aphid's host choices may, in the future, contribute to the development of sustainable management strategies.

Here we investigate the potential effects of volatile interactions between different undamaged barley cultivars on (i) the plant volatile emissions and (ii) consequences for aphid's olfactory attraction to host plants. We hypothesize that VOCs from emitting cultivars can induce changes of volatile emissions in receiving cultivars depending on the genotypic identity of the emitter and that such changes in olfactory cues can affect aphid host choice.

2 | METHODS

2.1 | Plants and insects

We used three different spring barley cultivars (*Hordeum vulgare* L.) in this study including: cultivars Salome (Nordaat Saatzucht GmbH), Fairytale and Anakin (Sejet Plant Breeding), since some of these cultivars in combination can decrease aphid plant acceptance in the laboratory and lower population size in the field (Dahlin et al., 2018; Kheam et al., 2023). All three cultivars have different pedigrees: Auriga × (Publican × Beatrix) for Salome, Colston × (Receipt × Power) for Fairytale, and (Tumbler × Response) for Anakin. Scandinavian Seed AB supplied seeds of the three cultivars. Before sowing, seeds were germinated between two filter papers in Petri dishes for 24 h at room temperature. Twelve seeds were sown per pot (9 × 9 × 7 cm), filled with P-soil (Hasselfors), and kept in the growing chamber for 9 days at 18–22°C, 50%–60% relative humidity, and L16:D8 h photoperiod.

The bird cherry-oat aphids (*Rhopalosiphum padi* L.), one of the most important pests in cereals were used as a model study insect. The wingless aphids used in the experiments were the fourth instar aphids and the adult aphids. The aphids were reared on oat (*Avena sativa* L.) cultivar Belinda in a separate growing chamber under the same growing conditions as the barley plants.

2.2 | Plant volatile exposure

Twin Perspex cages (Ninkovic, 2003) were used to explore the effects of volatile interactions between different barley cultivars on the receiving plant's volatile profiles and aphid's preference and repellency. In brief (Figure 1a), these cages are divided into two chambers—inducing and responding (each 10 × 10 × 40 cm), connected by a circular opening (7 cm diameter) in the middle wall. Air entered into the system through the chamber with an emitter plant and passed through the hole in the middle wall into the chamber with a receiver plant, before being vented outside the room. Airflow in the system was 1.2 L min^{-1}. Each individual potted plant was placed in a Petri dish to avoid the potential interactions between plants by root exudates. Plants were watered by an automated drop system (DGT Volmatic) for 2 min every day without adding extra fertilizers. The plants were placed in the exposing system at the one-leaf stage (7 days old). The plant exposure time was 5 days. Salome, the receiving cultivar, was exposed to Fairytale volatiles (SeF), Anakin volatiles (SeA), or clean air (Se0). Se0 was used as the absolute control (Dahlin et al., 2018; Kheam et al., 2023).

2.3 | VOCs collections

The impact of exposure on the VOC release of receiving Salome plants was investigated by gas chromatography–mass spectrometry (GC-MS). Headspace samples were collected from 12 plants per pot from unexposed Salome plants and Salome that were previously exposed to VOCs from Anakin or Fairytale, after 5 days of exposure (Figure 1a). Four replicates of each treatment were sampled in parallel per day and repeated three times resulting in 9–11 replicates per exposing treatment (unexposed Salome: $n = 9$, SeA: $n = 10$, SeF: $n = 11$). Pots with 12 barley plants were enclosed in polyethylene terephthalate oven plastic bags (35 × 43 cm; Melita) and these bags were baked in the oven for 2 h at 140°C before the volatile collection. The volatiles were collected with a push–pull system for 24 h. Charcoal-filtered air was pushed in to the oven bags with a flow of 600 mL min^{-1} while pulling the air out of the bags over an adsorbent trap with 400 mL min^{-1}. The VOCs were trapped on Tenax TA sample tubes (60/80 mesh size; GLScience) containing 80 mg adsorbent. 1-nonene was used as an internal standard for quality control purposes, by injecting 20 ng on the top of the collection tube mesh right before headspace collections. Volatiles were released from the adsorbent tubes by thermal desorption with an Optics 3 Injector (GLScience) at 250°C. The thermal desorbed compounds were separated using an Agilent 7890 N GC system equipped with an HP-1MS capillary column (30 × 0.25 mm id × 0.25 µm film thickness, 100% dimethylpolysiloxane) coupled to an Agilent 5975C mass spectrometer (Agilent Technologies Inc.). Injection was employed using helium as carrier gas (Helium 6.0) with a flow of 1.3 mL min^{-1}. The GC temperature programme was as follows: Initial oven temperature of 30°C was held for 2 min, increased at a rate of 5 K min^{-1} to 150°C, followed by a rate of 10 K min^{-1} to the final temperature of 250°C and held for 15 min. The GC inlet line temperature was 250°C, and the ion source temperature was 180°C. The quadrupole mass detector was operated in the electron impact mode at 70 eV, MS gain was set to 10. All data were obtained by collecting the full-scan mass spectra within the range of 40–500 *m*/*z*.

2.4 | Volatile analyses

The volatile compounds from the chromatograms were identified and quantified with the 'Automated Mass Spectral Deconvolution and Identification System' (V. 2.71; National Institute of Standards and Technology) according to (Gross et al., 2019). The settings for deconvolution were set to medium sensitivity and resolution, and a high shape requirement with a component width of 32 and one adjacent peak subtraction. Identification criteria were applied as follows: match factor ≥ 80% with relative retention index deviation ≤ 5% + 0.01 from the reference value. The match factor penalty was set to very strong with a maximum penalty of 25. A signal-to-noise ratio filter of <300 was applied. Only compounds where identities were confirmed by the analysis of authentic standards were reported as identified.

2.5 | Olfactory bioassay with barley plants

A two-way airflow olfactometer was used to measure the olfactory responses of aphids. The olfactometer consisted of two stimulus zones, in two arms directly opposite to each other connected by a

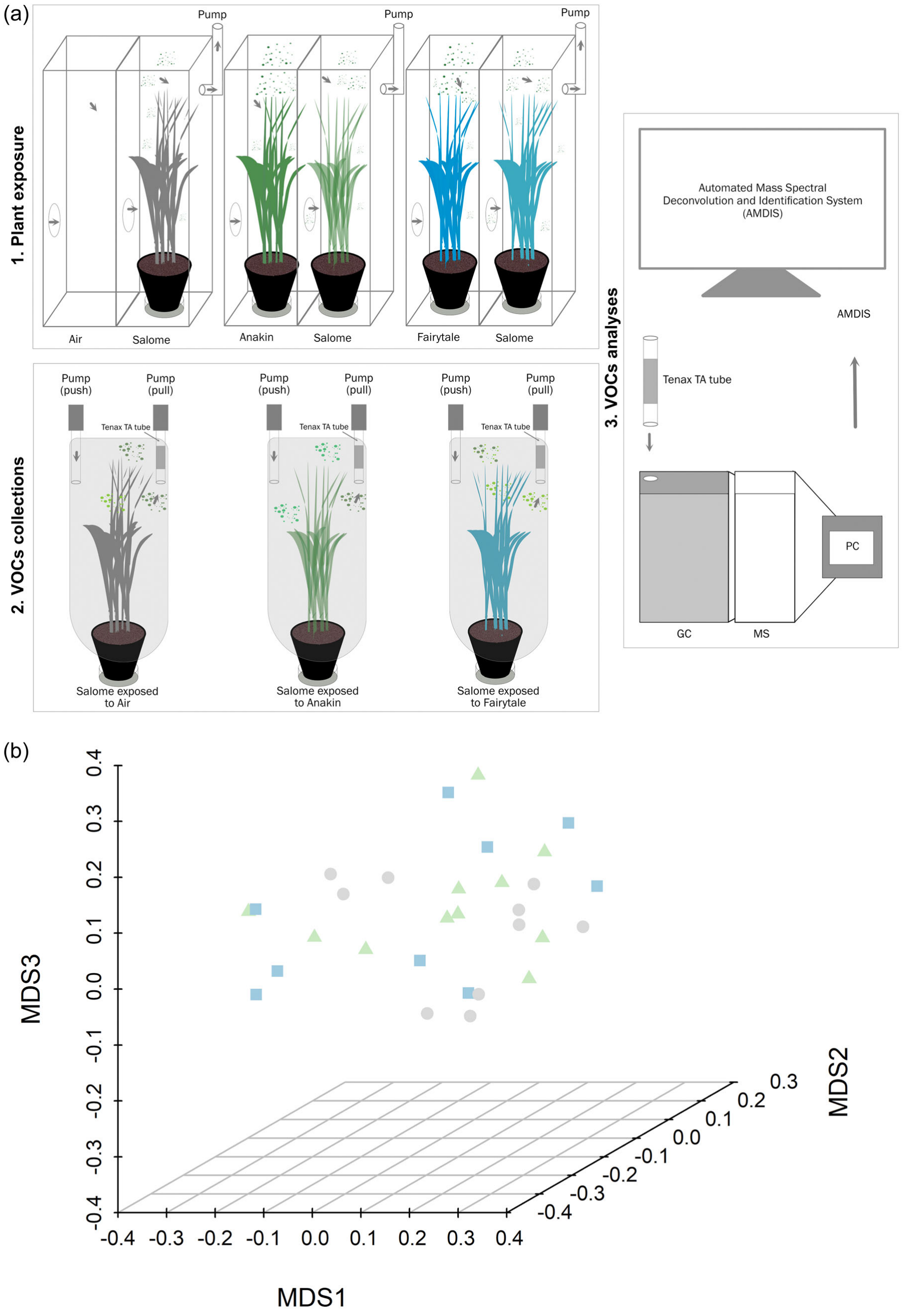

FIGURE 1 (See caption on next page).

neutral central zone separating them. The airflow of 180 mL min^{-1} was set in the olfactometer to ensure discrete air currents in the side zones (Dahlin et al., 2015; Ninkovic et al., 2013; Tous-Fandos et al., 2023).

We conducted three olfactory experiments to compare aphid preferences and repellency for different plant odours. We first compared the aphid's cultivar preferences by offering the volatiles from (a) Fairytale versus Salome, and (b) Anakin versus Salome by offering odours from both cultivars simultaneously against each other (Figure 4a). Second the aphid's preferences for odours from previous exposed plants: (a) SeF versus a Se0, and (b) SeA versus Se0 - (Figure 4c). Finally, we investigated the potential repellency of odours from previously exposed plants: (a) SeF, and (b) SeA when offered against air from an empty cage (0) (Figure 4e). The arms of the olfactometer were connected to the odours sources from the two-chamber cages containing plants. The sucking pump and air flowmeters were used to control the airflow and provide a consistent flow of 180 mL min^{-1} in all olfactometers. We used different sets of plants in each experiment.

The wingless aphids were randomly collected from the stock cultures using a fine paintbrush and placed in Petri dishes with moist filter paper to prevent dehydration. The aphids were then left in the experiment room for about 30 min to acclimatize before the experiments. A single wingless aphid was introduced into the central zone of the olfactometer through a hole on the top and after an adaptation period of 10 min the position of the aphid in the olfactometer was recorded at 3 min intervals over a 30 min period. One aphid was tested once. A clean olfactometer was used for each aphid. The olfactometers were cleaned with 10% Teepol L (TEEPOL) and rinsed with 70% ethanol solution and distilled water and left to air dry. To avoid the influence of lighting conditions outside the laboratory on aphid behaviours, the olfactory experiments were conducted in a dark room under artificial light (Osram FQ80W/840 HO Constant Lumilux Cool White (4000 K)) at 60 µmol m^{-2} s^{-1} above the olfactometer. These experiments were carried out between 9:00 and 17:00 during the day. The average room temperature was about 20°C with the 40%–50% of relative humidity.

The total number of visits of a single aphid per a single arm after 10 recordings was regarded as one replicate. If an aphid did not move for longer than 10 min, these individuals were discarded and not included in the analyses. The aphid visits into middle (central) zone were not taken for the analyses. Data were expressed as the total of individual aphid visits per olfactometer arm during the observation period. Each comparison was replicated with 20 to 24 individuals.

2.6 | Olfactory bioassay with *trans*-β-ocimene compound

To confirm the olfactory responses of aphids to the more abundance volatile in SeF, the dose olfactory responses of *trans*-β-ocimene experiments were conducted by using serial dilutions based on the quantified amount in the headspace. *Trans*-β-ocimene (≥99% purity; Toronto Research Chemical Inc.) was diluted to five different concentrations (0.01, 0.1, 1, 10 and 100 ng µL^{-1}) in methanol. Due to the dynamic characteristics of plant volatile emission over diurnal and ontogenetic time (Schuman et al., 2016), these five concentrations were tested in the olfactometer experiments to determine the effects of *trans*-β-ocimene and its relevant concentrations on aphid preference. Aphid olfactory responses to the different concentrations of *trans*-β-ocimene were tested against methanol as a control (Figure 5a). Each diluted concentration of *trans*-β-ocimene and methanol was dosed at a volume of 10 µg on small pieces of filter paper, allowed to evaporate for 1–2 min and placed into glass tubes (2.5 mm diameter) connected to holes in the sides of the olfactometer arms. The observation and experiment protocol is the same as the testing aphid responses to barley plant's odour. Each pair comparison was replicated with 19–22 individuals.

2.7 | Statistical analyses

The Olfactory response and volatile profile data were analyzed by using R statistical software (R Core Team, 2022). Data visualization was realized using functions from ‘ggplot2’ package (Wickham, 2016).

2.7.1 | Aphid olfactory responses

Wilcoxon matched-pairs test was used to analyze the pair-choice test for aphid olfactory responses.

FIGURE 1 The overall composition of volatile emissions from Salome is not different after exposing to other emitter cultivars or air. (a) Volatile collection process. 1. Plant exposure: Before the volatile collection, one pot with 12 barley plants (9-day) was exposed to volatiles from other barley cultivars or to air for 5 days. The exposures included Salome exposed to air (Se0), Salome exposed to Anakin (SeA) and Salome exposed to Fairytale (SeF). 2. Volatile organic compound (VOC) collection: Push–pull volatile collections of plant headspace were taken from unexposed Salome plants and Salome plants that were previously exposed to VOCs from Anakin or Fairytale. 3. VOC analyses: Thermal desorption–gas chromatography (GC)–mass spectrometry (MS) was used for chemical analysis of plant VOCs followed by identification and quantification with ‘Automated Mass Spectral Deconvolution and Identification System’. (b) Nonmetric multidimensional scaling (NMDS) plot visualizing Bray–Curtis dissimilarities calculated from proportional VOC compositions emitted from barley plants. Each point represents the volatile profile emitted from 12 cv. Salome after 5 days exposure to air (unexposed = grey circles, *n* = 9), to VOCs from cv. Anakin (green triangles, *n* = 10) or VOCs from cv. Fairytale (blue squares, *n* = 11); 3-day stress: 0.119.

2.7.2 | VOCs of profile compositions

To calculate and visualize differences in plant VOC compositions we used the 'vegan' package (Oksanen et al., 2022). Nonmetric multidimensional scaling plots were used to visualize Bray–Curtis dissimilarities of the VOC composition from Salome plants after exposure to air or volatiles from Anakin or Fairytale calculated using the *metaMDS* function. Wisconsin double standardization was used for scaling. Permutational multivariate analysis of variance (PERMANOVA) was used to test for discrimination between differently exposed plant volatile profiles. Bray–Curtis distance matrix was calculated with *vegdist* function and was used for permutation testing using *adonis2* function with 10 000 permutations. The sampling day was included as strata to preserve the effect of the sampling day.

2.7.3 | Fold change of single compounds

The peak area per gram dry weight was used to calculate the fold change in the emission of single volatile compounds. Therefore, the mean peak area per gram dry biomass from unexposed plants was calculated per day, accounting for daily variation. The logarithmic ratio of the peak area per dry weight from each exposed plant sample to the mean of unexposed plants from the corresponding day was calculated as

$$\text{Fold-change} = \log\left(\frac{\text{exposure}_{\text{day}[i]}\left(\frac{\text{peak area}}{\text{DW}}\right) + 1}{\text{mean}\left(\text{control}_{\text{day}[i]}\left(\frac{\text{peak area}}{\text{DW}}\right) + 1\right)}\right). \quad (1)$$

To visualize the change of the emission of single compounds from Salome induced by exposure to different cultivars a heatmap was generated based on the mean fold-change. Generalized linear models with Gamma (log link) distributions were fitted to compare the fold-change between Anakin and Fairytale exposed Salome plants. The identity of the emitter cultivar and the sampling day were fitted as fixed effects. The model fit was visually inspected as recommended by Zuur et al. (2010). Differences in fold-change of single compounds from Salome plants exposed to Anakin or Fairytale were calculated using estimated marginal means and 95% confidence intervals with the *emmeans* function from the 'emmeans' package (Length, 2022). Additionally, the peak are per g dry weight of *trans*-β-ocimene was analysed in the same way.

3 | RESULTS

3.1 | Volatile emissions of host plants after exposure

To identify changes in the volatile emission of Salome plants after exposure to VOCs from Fairytale, Anakin or air, the headspace of previously exposed Salome plants was collected and analyzed via GC. In total, 62 components were analyzed from the Salome headspace samples. The exposure to air or VOCs from the other barley cultivars did not lead to a differentiation of the overall composition of VOCs released from Salome (Figure 1b, PERMANOVA, $F = 0.51$, $p = 0.58$, $n = 30$).

As changes in the release of single compounds, or rather, shifts in specific blends of plant VOCs can be important for host recognition of insects, induced fold changes of single volatile compounds were analyzed and visualized as heatmap (Figure 2). Only three compounds, *trans*-β-ocimene and two unidentified compounds (RI 855 and RI 1093) changed significantly different after exposure to Fairytale or Anakin VOCs (Figure 2). *Trans*-β-ocimene, RI 855 and RI 1093 were statistically increased in SeF compared to SeA (Figure 3b,c, general linear model [GLM], Estimate = 6.27, SE = 2.59, $p = 0.02$; Estimate = 0.51, SE = 0.19, $p = 0.01$; Estimate = 0.02, SE = 0.01, $p = 0.04$), respectively. Comparing the peak area per biomass (g dry weight) of *trans*-β-ocimene released from unexposed and exposed Salome plants, SeF was significantly higher in *trans*-β-ocimene compared to Salome exposed to air (Figure 3a, GLM, Estimate = 4.47, SE = 2.22, $p = 0.01$). No differences in the peak area of *trans*-β-ocimene were detected between SeA and Se0 (Figure 3a, GLM, Estimate = 2.29, SE = 1.16, $p = 0.25$) and between SeF and SeA (Figure 3a, GLM, Estimate = 0.51, SE = 0.24, $p = 0.36$).

3.2 | Olfactory preference of *R. padi* for barley plants

A series of olfactory experiments were conducted to assess the preferences of *R. padi* for odours from individual unexposed cultivars and exposed Salome plants. We first tested whether *R. padi* has a general preference for cv. Salome or the cultivars used as emitters (Anakin and Fairytale), odours of unexposed plants were offered simultaneously in olfactometer trials. *R. padi* individuals did not show an olfactory preference for Anakin over Salome (Figure 4b, Wilcoxon, $V = 63$, $p = 0.53$, $n = 20$), nor Fairytale over Salome (Figure 4b, Wilcoxon, $V = 50.5$, $p = 0.37$, $n = 20$).

To confirm with the volatile emission changes in plant exposure results, the impact of exposure to volatiles from other cultivars (plant–plant volatile interaction) on the olfactory attraction of *R. padi* for Salome plants was then investigated. Aphids were significantly less attracted to the odours of Salome plants that were previously exposed to Fairytale VOCs than unexposed Salome plants (Figure 4d, Wilcoxon, $V = 127.5$, $p = 0.01$, $n = 24$). Aphids did not show a preference for SeA VOCs over unexposed Salome plants when offered simultaneously (Figure 4d, Wilcoxon, $V = 40.5$, $p = 0.09$, $n = 24$).

We additionally evaluated the avoidance responses for *R. padi* to the odours of previously exposed Salome plants. Aphids significantly preferred air over odours from Salome exposed previously to Fairytale VOCs (Figure 4f, Wilcoxon, $V = 131$, $p = 0.04$, $n = 20$). In contrast, odour of Salome exposed previously to Anakin VOCs were more significantly attractive for *R. padi* individuals than air (Figure 4f, Wilcoxon, $V = 39.5$, $p = 0.04$, $n = 22$).

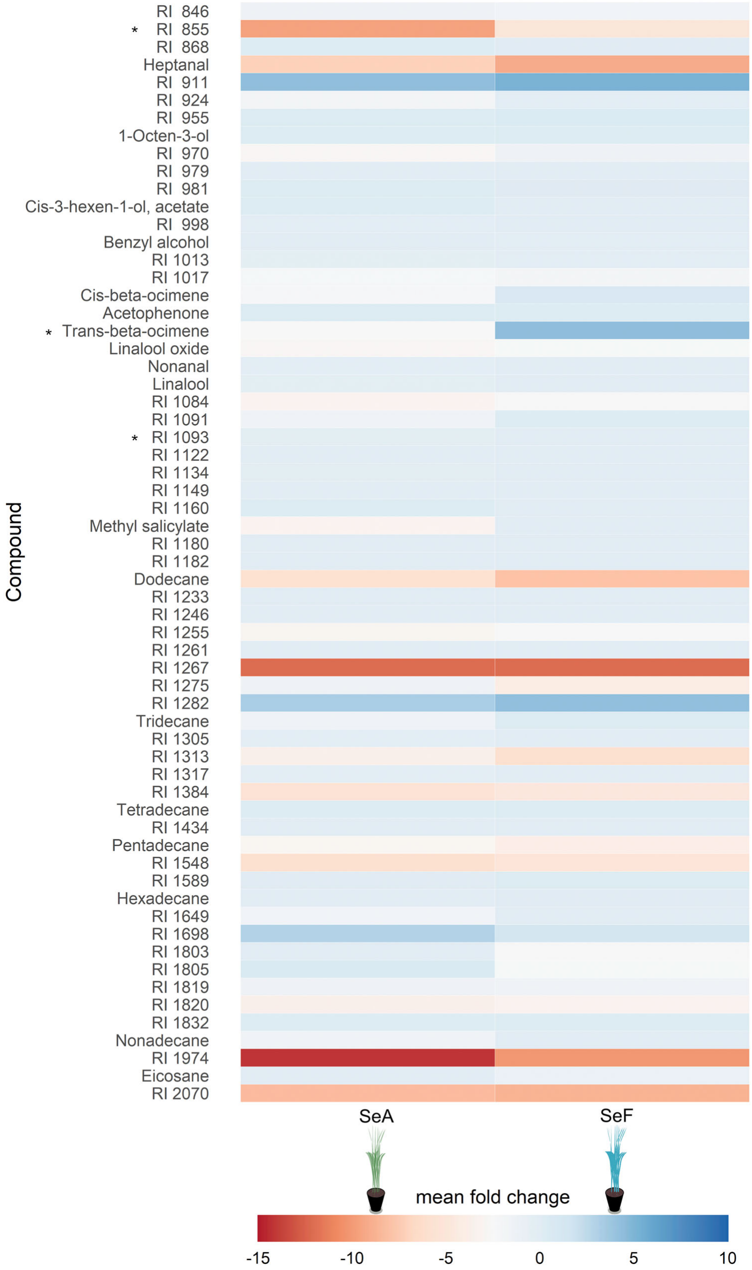

FIGURE 2 (See caption on next page).

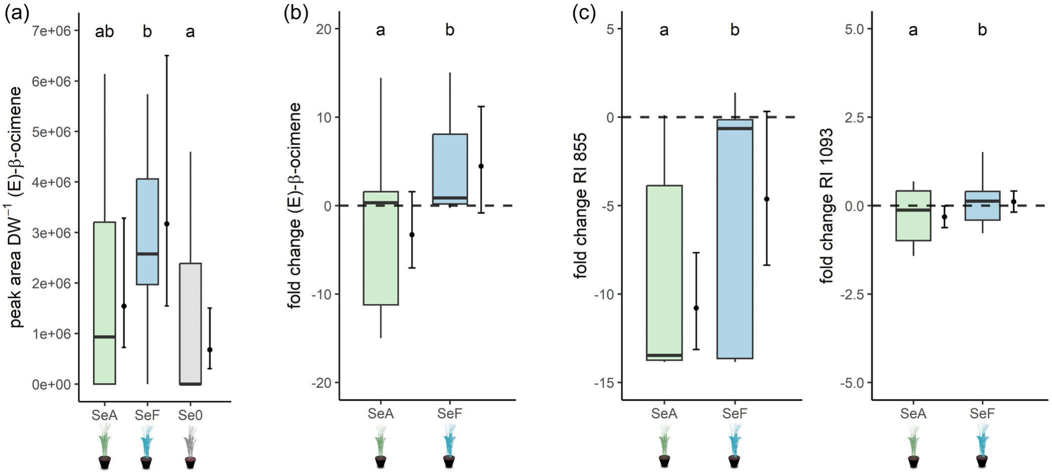

FIGURE 3 Exposure to Fairytale volatile organic compounds (VOCs) increases *trans*-β-ocimene, RI 855 and RI 1093 released from Salome. (a) Amount (peak area) of *trans*-β-ocimene per biomass (g dry weight) released from Salome after exposure to Anakin (SeA), Fairytale (SeF), or air (Se0). (b) Fold change in *trans*-β-ocimene, (c) RI 855 and (d) RI 1093 release from Salome in response to Fairytale or Anakin VOC exposure. Boxes represent the interquartile range (IQR) and whiskers extend to 1.5 × IQR. Bars represent the 95% confidence intervals with the estimated marginal means (EMMs) as dots, obtained from generalized linear models fitted with Gamma (log link) distributions. Letters indicate statistical differences between EMMs of groups at the 0.05 significance level. [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Olfactory preference of *R. padi* for pure *trans*-β-ocimene compound

To confirm whether *trans*-β-ocimene has an impact on aphid behaviour, we performed a series of olfactory experiments with five different concentrations of *trans*-β-ocimene (0.01, 0.1, 1, 10 and 100 ng μL^{-1}) against Methanol (solvent control). No difference was detected at lower concentration of *trans*-β-ocimene (0.01, 0.1 and 1 ng μL^{-1}) compared to methanol (Figure 5b, Wilcoxon, $V = 78.5$, $p = 0.32$, $n = 22$; $V = 45$, $p = 0.23$, $n = 22$; $V = 52.5$, $p = 0.15$, $n = 20$), respectively. At the two highest concentrations (10 and 100 ng μL^{-1}), *R. padi* significantly preferred the control arm and avoided the *trans*-β-ocimene (Figure 5b, Wilcoxon, $V = 49.5$, $p = 0.03$, $n = 22$; $V = 31.5$, $p = 0.01$, $n = 19$), respectively.

4 | DISCUSSION

Our findings demonstrate that volatile interactions between specific undamaged cultivars can enhance the emission of certain volatile compounds in receiving plants, resulting in the disruption of aphid olfactory responses. The phenomenon is context specific, depending on the genotypic identity of the emitting plants. This study, therefore, highlights the significant roles of VOCs in plant–plant communications, affecting plant–insect interactions in cultivar mixture cropping systems.

4.1 | Airborne interactions between undamaged plants alter volatile emission of receiving plants

Plants have the capacity to detect and respond to the surrounding environments by chemical cues, including emitted volatiles from neighbouring plants (Brosset & Blande, 2022; Ninkovic et al., 2016). Constitutively emitted VOCs contain unique information about genotype identity (Dahlin et al., 2018), which receiving plants can detect and use to identify neighbours, then respond through growth adaptation (Ninkovic, 2003). It has been previously shown that the three individual cultivars examined in this study (Salome, Anakin, and Fairytale) had distinct constitutively produced volatile emissions (Dahlin et al., 2018). Our fold change analyses highlighted that the

FIGURE 2 Exposure to emitter volatiles induces changes in the volatile organic compound (VOC) emission of receiver. Heatmap visualization of the mean values of fold change of single compounds emitted from Salome exposed to Anakin (SeA) ($n = 10$) and Salome exposed to Fairytale (SeF) ($n = 11$). The fold change value represents the logarithmic ratio of the peak area per dry weight from each exposed plant sample to the mean of unexposed plants. The scale ranges from dark red, indicating a strong decrease of VOC emission to a highly increased (dark blue) emission of the compound in response to exposure to VOCs from Anakin or Fairytale relative to the emission from unexposed Salome plants. Asterisks(*) indicate significant changes from GLM analyses ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

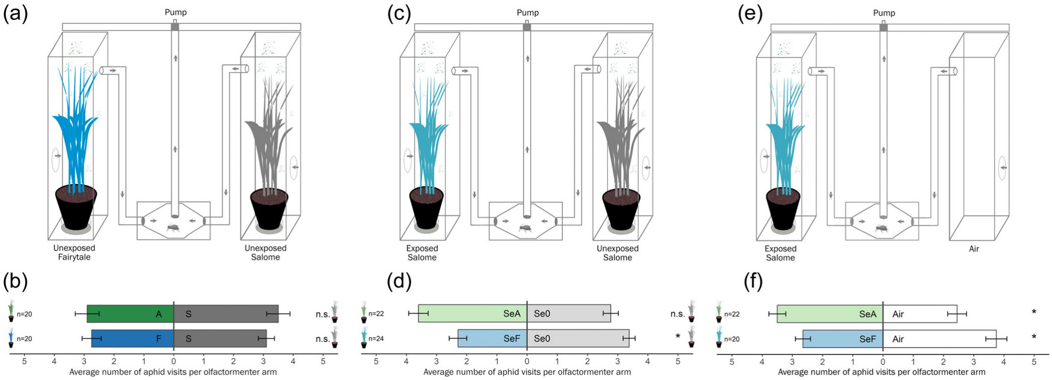

FIGURE 4 Aphid shows repellent effects from odours in Salome exposed to Fairytale (SeF) against air and less attraction in Salome exposed Fairytale (SeF) against Salome exposed to air (Se0). Before the olfactory experiment, one pot with 12 barley plants (9-day) was exposed to or not exposed to other barley cultivars as emitter for 5 days. The exposed/unexposed pot with 12 barley cultivar (14-day) was placed in each cage connected to an olfactometer and a suction pump that was used to facilitate airflow from the plants through the olfactometer. A wingless adult aphid was placed in the middle of the olfactometer. (a) Aphid preference test between individual cultivars: unexposed Fairytale (emitter) against unexposed Salome (receiver). (b) Aphid preference test on odours between individual cultivars: unexposed Anakin (A) against unexposed Salome (S) (*n* = 20), and unexposed Fairytale (F) against unexposed Salome (S) (*n* = 20). (c) Aphid preference test between exposed Salome against unexposed Salome as control. (d) Aphid preference test on odours between receiving cultivars: SeA against Se0 (*n* = 22), and SeF against Se0 (*n* = 24). (e) Aphid avoidance test between exposed Salome cultivar against Air. (f) Aphid avoidance test on odours between receiving cultivars against air: SeA against Air (*n* = 22), and SeF against Air (*n* = 20). Error bars indicate the standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

emission of *trans*-β-ocimene and two unidentified compounds were significantly increased in Salome exposed Fairytale, but not when Salome was exposed to Anakin. It, therefore, seems that Salome can distinguish between different neighbouring cultivars by perceiving volatiles and activating specific biological pathways, altering its own volatile emissions in response. To our knowledge, this study presents the first evidence that volatiles released by specific undamaged cultivars can prompt neighbouring cultivars to alter their physiological state by emitting greater quantities of specific volatile compounds. This highlights the significant involvement of VOCs in interactions between undamaged plants.

Plants discriminate between the emitted volatiles of different neighbours (Kigathi et al., 2019; Ninkovic et al., 2016, 2020). In studies of interactions between different plant species, Ninkovic et al. (2013) revealed that volatile compounds from undamaged onions can enhance the release of certain volatile compounds from receiving potatoes. Ninkovic (2003) also showed that exposure to specific cultivars could alter the pattern of biomass allocation for receiving plants, away from leaves and in favour of roots. As a result, these receiving plants become more competitive for limited nutrition and water resources. Given the evidence from these previous studies and our own, it is clear that plants are capable of detecting, responding to, and distinguishing between neighbouring plants, depending on the identity of the emitters. The identification of a competitive neighbour can therefore lead to specific morphological and physiological adaptations to better tolerate resource competition.

While the mechanisms of VOC perception in plants are not well understood, the effects are context-dependent (Hemachandran et al., 2017). The observed rise in specific volatile emissions in receiving plants may be attributed to active mechanisms of volatile-mediated plant–plant interactions, which encourage specific physiological pathways, such as induced or primed defenses. In this case, the observed increased emission of certain volatiles in our study could possibly be a mechanism to induce or prime defenses in receiving plants. Another possible mechanism, which cannot be excluded at this stage, is a passive one: the adhesion of volatiles to the surface of recipient plants, which could be re-emitted later (Li & Blande, 2015). Therefore, further research is needed to uncover the underlying mechanisms of the observed effects between specific undamaged plants.

4.2 | Aphid olfactory responses to specific airborne-induced plant volatiles

Plant volatiles are olfactory cues that can be detected and utilized by insects in host plant location and selection (Bruce et al., 2005). In line with the emitter-specific impact on the VOC emission of receiving Salome plants, *R. padi* showed emitter-dependent preferences for differently-exposed Salome plants. While aphids were less attracted to Salome after exposure to Fairytale, compared to Se0, no preference was detected when offering Salome previously exposed

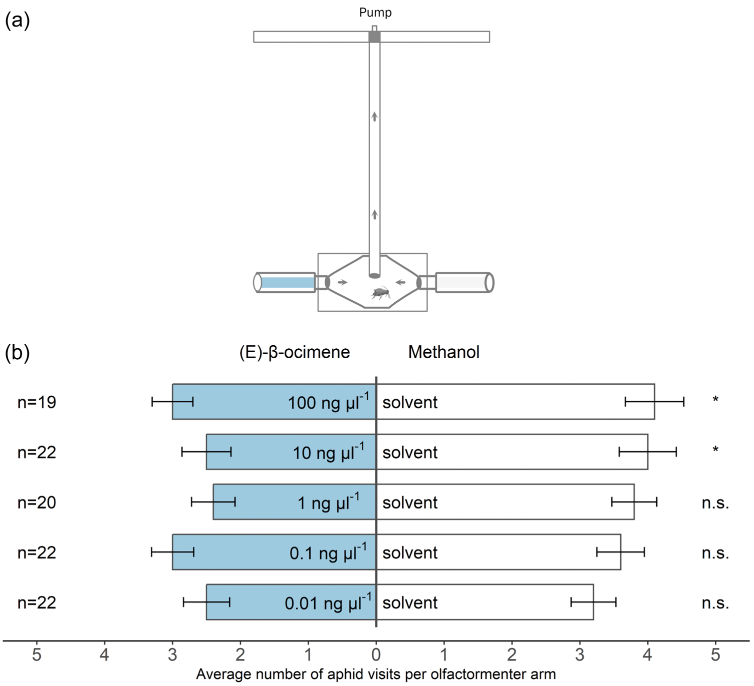

FIGURE 5 *Trans*-β-ocimene repels *Rhopalosiphum padi*; aphid shows repellent effects from *trans*-β-ocimene (≥ 10 ng μL^{-1}) against solvent (methanol). (a) Aphid avoidance setup. Two-arm olfactometer was used: The light blue colour represents the arm of *trans*-β-ocimene and the grey colour represents the arm of solvent as control (methanol). Five series of *trans*-β-ocimene dilutions (0.01, 0.1, 1, 10 and 100 ng μL^{-1}) against solvent were tested in olfactometer. Each 10 μg of each dilution concentration was placed on a small piece of filter paper in each glass tube connected to an olfactometer and a suction pump that was used to facilitate airflow from the glass tube through the olfactometer. A wingless adult aphid was placed in the middle of the olfactometer. (b) Aphid avoidance tests were conducted on *trans*-β-ocimene at five different concentrations against solvents: 0.01 ng μL^{-1} against solvent (n = 22), 0.1 ng μL^{-1} against solvent (n = 22), 1 ng μL^{-1} against solvent (n = 20), 10 ng μL^{-1} against solvent (n = 22) and 100 ng μL^{-1} against solvent (n = 19), respectively. Error bars indicate the standard error of the mean. The n.s. represent no significant differences and asterisks(*) represent significant differences according to the Wilcoxon signed-rank test ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

to Anakin or to air. This highlights that the changes in volatile emissions induced by certain emitters can impact the behavioural responses of herbivorous insects. Accordingly, Tous-Fandos et al. (2023) showed that the alternation in the odour profile of specific wheat cultivar mixtures affects the host preference of aphids.

The reduced attraction and increased avoidance of aphids to the host plant odour could be due to the effects of specific chemical compounds (Webster et al., 2010). *trans*-β-ocimene, emitted at higher levels in SeF, induced a significant avoidance response in aphids, indicating the potential role of *trans*-β-ocimene in aphid host preference. The olfactory responses of aphids to the other two unidentified compounds remain unknown. Further chemical identification is needed to discern the roles of these compounds as cues for aphid detection and the location of their host plants. Nonetheless, our findings support the hypothesis that aphids can respond to particular compounds at specific concentrations, influencing their search for a host plant (Webster, 2012; Webster et al., 2010).

Trans-β-ocimene is known to be a HIPV that effectively repels insect pests (Thompson et al., 2022). Externally applying β-ocimene can trigger induced defense in recipient plants, resulting in subsequent adverse effects on the olfactory responses, settling, feeding behaviours and overall performance of aphids (Cascone et al., 2015; Kang et al., 2018). Interestingly, exposure to the volatiles of a specific undamaged cultivar can enhance *trans*-β-ocimene production in undamaged plants as well. As *trans*-β-ocimene has repulsive effects on aphids, it may serve as a relevant compound for sustainable pest control in crop production.

The specific ratios of each VOC could contribute to aphid behavioural responses (Dardouri et al., 2019), and certain compound blends could be more repellent than single VOCs (Bruce & Pickett, 2011; Dardouri et al., 2019; Deletre et al., 2016). As not all volatile compounds used by *R. padi* for host plant detection are currently identified, we compared the composition of all detected plant volatiles, which showed no significant difference in response to the exposure. However, the shifts in emissions of specific combinations of VOCs influential to *R. padi* behavioural activity can be relevant for the observed olfactory preferences.

4.3 | Ecological aspects of cultivar mixtures in pest control

This study provides fresh knowledge of the potential specificity of volatile-mediated plant–plant interactions, affecting plant–insect interactions and highlighting the significant roles of VOCs in pest control. Our results support the hypothesis that plants can use emitted volatiles from specific neighbouring plants to prepare for growth adaptation, future threat or stress (Ninkovic et al., 2020). Aphids, like many other herbivorous insects, use plant volatiles as olfactory cues to make crucial decisions regarding host plant selection, feeding and overall development (Pettersson et al., 2017). The volatile components of plants carry information about their quality for the aphids. The observed repellent effects on aphids in certain cultivar mixtures suggest that aphids struggle to find suitable host plants in these instances. This could reduce the establishment of new aphid colonies due to prolongation of searching for host plants and increased exposure to natural enemies in the agroecosystem. Volatile interactions between undamaged Salome plants exposed to Fairytale can induce responses in receiving plants which disrupt aphid feeding and reduce their performance (Kheam et al., 2023). In the field, the reduction of aphid populations observed in specific cultivar mixtures, such as Salome–Fairytale mixtures (Dahlin et al., 2018), is consistent with the mechanistic explanation proposed from the results of our laboratory experiments. All of these findings indicate that volatile interactions between plants constitute a potential underlying mechanism of insect pest suppression in cultivar mixtures and, on a broader scale, suggest their potential in pest management to enhance the sustainability of cropping systems.

5 | CONCLUSION

Plants detect VOCs emitted by their specific, undamaged neighbours and respond by altering their own volatile emissions, thus enhancing their defenses. Our results suggest that volatile interactions in specific cultivar mixtures have a major implication for plant–insect interactions and bear potential as an effective approach for the development of integrated pest management in crop protection. The observed increasing *trans*-β-ocimene in plant–plant communication and its repellent effects on insect pests suggest this compound is a suitable candidate for sustainable agricultural pest control. Currently, control measures for insect pests by cultivar mixtures are still limited, but exploring volatile-mediated plant–plant communications to understand interactions among plants, insect pests, natural enemies, and diseases will help us determine the novel points of control that will open the door for wider adoption of cultivar mixtures in sustainable agricultural practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the correspondence authors, without undue reservation.

ORCID

Sokha Kheam http://orcid.org/0000-0002-4918-3544
Jannicke Gallinger http://orcid.org/0000-0003-0179-1791
Velemir Ninkovic http://orcid.org/0000-0001-9276-7169

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ORIGINAL PAPER

Volatile interactions between specific undamaged barley cultivars affect aphid feeding behavior and performance

Sokha Kheam[1,2] · **Dimitrije Markovic**[3,4] · **Diana Rubene**[3] · **Saveng Ith**[2] · **Velemir Ninkovic**[1]

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Abstract

Recent studies have demonstrated that cultivar mixtures can reduce aphid plant acceptance and population development. It is still unknown as to which underlying mechanisms may contribute to this phenomenon. We investigated the effects of volatile interactions between undamaged barley cultivars on aphid feeding behavior and performance in the laboratory. Spring barley (*Hordeum vulgare* L.) cultivar Salome was exposed to volatiles from Fairytale (SeF), Anakin (SeA), or clean air (Se0). We used an electrical penetration graph to test the effect of exposure to neighbor volatiles on the feeding behavior and performance of bird cherry-oat aphids (*Rhopalosiphum padi* L.). We also assessed aphid relative growth rate, intrinsic rate of increase, and development time on exposed and unexposed Salome plants. Aphids spent significantly longer time on epidermis and mesophyll plant tissues on SeF than Se0, and no difference was observed between SeA and Se0. Significant decreases in the duration of phloem ingestion and phloem sustained ingestion were recorded in SeF showing that volatile-induced effects cause difficulty for aphids to feed. However, no differences in these variables were detected between SeA and Se0. We also observed reduced aphid relative growth rate and intrinsic rate of increase on SeF compared to Se0 and SeA. Our study demonstrated that, in a specific combination, exposure of one barley cultivar to volatiles from another one can change aphid feeding behavior and performance, probably due to changes in host plant properties/quality. Our results provide an insightful explanation of mechanisms responsible for the reduced aphid population development previously observed in the field.

Keywords Variety mixture · Plant–plant interactions · Plant–insect interactions · Plant protection · Induced defense · EPG

Key messages

- Volatile interactions between undamaged barley cultivars disrupt aphid feeding behavior and reduce aphid relative growth rate and intrinsic rate.
- Volatile-induced responses in plants, which affect aphid feeding and performance, depend on the genotype of the neighboring cultivar.
- Volatile interactions between specific cultivars could be the underlying mechanism, which reduces aphid population development in cultivar mixtures in the field.

Introduction

Plant diversity contributes to ecosystem stability (Prieto et al. 2015; Isbell et al. 2017), while in agroecosystems, botanical diversity can reduce damage by insect pests, improve biological pest control and increase food production (Ratnadass et al. 2012). Combining different cultivars in mixtures to increase within field diversity has been suggested as a promising strategy to reduce pest pressure (Tooker and Frank 2012; Koricheva and Hayes 2018; Snyder et al. 2020). The quality of evidence for pest suppression in

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✉ Velemir Ninkovic
velemir.ninkovic@slu.se

[1] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2] Department of Biology, Royal University of Phnom Penh, Phnom Penh, Cambodia

[3] Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[4] Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

cultivar mixtures is varied, as some studies show that cultivar mixtures can reduce aphid population sizes (Shoffner and Tooker 2013; Snyder et al. 2020), while others report the lack of effects on aphids (Mansion-Vaquié et al. 2019; Grettenberger and Tooker 2020). Understanding these inconsistent effects of cultivar mixtures on aphids is important from both an ecological and practical perspective and could be achieved by clarifying the underlying mechanisms responsible for reduced aphid abundance.

Plant species diversity or genotypic diversity could affect pest insects via several mechanisms including dilution effect, abundance of natural enemies, and associational resistance. The dilution effect leads to reduced pest population spread via increased host plant finding time (Malézieux et al. 2009; Hambäck et al. 2014). The natural enemy hypothesis predicts that diverse plant communities host a higher abundance of natural enemies, which could suppress herbivorous pests (Cook-Patton et al. 2011). The cultivar mixtures can attract more natural enemies than pure stands (Ninkovic et al. 2011). Associational resistances involve specific plant associations that provide physical and chemical barriers that suppress insect pests (Malézieux et al. 2009; Dahlin et al. 2018). Volatile interactions between undamaged neighboring plants via changes in receiving plant physiology can potentially present one of the underlying mechanisms for reduced aphid performance (Ninkovic et al. 2016).

Volatile organic compounds (VOCs) play a major role as cues and signals in trophic interactions (Ninkovic et al. 2020), and can stimulate or prime defense responses in neighboring plants (Heil and Karban 2010; Brilli et al. 2019). Herbivore-induced plant volatiles (HIPVs) induce changes in the neighboring plants directly and indirectly, which can both lead to pest suppression and attraction of their natural enemies (Ninkovic et al. 2001; Dicke and Baldwin 2010; War et al. 2011; Karban et al. 2014). Volatile interactions between undamaged plants can also change the physiology of the receiving plants with subsequent influence on organisms through higher trophic levels (Ninkovic et al. 2006). This phenomenon, known as allelobiosis (Pettersson et al. 2003), could potentially be responsible for aphid suppression in cultivar mixtures. For instance, Dahlin et al. (2018) reported that mixing specific barley cultivars significantly reduced aphid population development in a field trial, while volatile interactions in the same cultivar combinations reduced aphid plant acceptance in a laboratory test. This clearly indicates that volatile interactions between specific cultivars can affect the early stages of aphid establishment in plants.

We hypothesized that the genetic identity of emitting cultivar expressed through their specific volatile profile plays an important role in the induction of defense responses in receiving cultivar and affect aphid performance. The primary aim of this study was to investigate the effect of volatile interactions between specific barley cultivars on (i) aphid feeding behavior, (ii) aphid relative growth rate, intrinsic rate, and development time on receiving cultivar after exposure to volatiles from another cultivar, and (iii) test whether aphid responses are dependent on specific cultivar used as an emitter.

Materials and methods

Plants and insects

Spring barley (*Hordeum vulgare* L.) cultivars Salome (Nordaat Saatzucht GmbH, Germany), Fairytale and Anakin (Sejet Plant Breeding, Denmark) were used in this study, because some of these cultivars in combination can reduce aphid plant acceptance in the laboratory and lower population size in the field (Dahlin et al. 2018). The pedigrees of the cultivars were Auriga × (Publican × Beatrix) for Salome, Colston × (Receipt × Power) for Fairytale and Tumbler × Response for Anakin. These cultivars were obtained from Scandinavian Seed AB, Linköping, Sweden. Before sowing, seeds were germinated in Petri dishes between two filter papers for 24 h at room temperature. One seed was sown per pot (9 × 9 × 7 cm), filled with P-soil (Hasselfors, Sweden), and kept in the growing chamber for 9 days at 18–22 °C, 50–60% relative humidity, and L16:D8 h photoperiod.

As a model insect, we used the bird cherry-oat aphid (*Rhopalosiphum padi* L.), which is one of the most important pests in cereals. The aphids used in this experiment were reared on oat (*Avena sativa* L.) cultivar Belinda in separate growing chambers under the same growing conditions as for the plants.

Plant volatile exposure

To study the effects of volatile interactions between different barley cultivars on aphid feeding behavior and performance, we used twin Perspex cages (Ninkovic 2003). These cages are divided into two chambers–inducing and responding (each 10 × 10 × 40 cm), connected by a circular opening (7 cm diameter) in the middle wall. Air entered into the system through the chamber with an emitter plant and passed through the hole in the middle wall into the chamber with a receiver plant, before being vented outside the room. Airflow in the system was 1.2 L/min. Each individual potted plant was placed in a Petri dish to avoid the potential interactions between plants by root exudates. Plants were watered by an automated drop system (DGT Volmatic) for 2 min every day without adding extra fertilizers.

The plants were placed in an exposing system at the one-leaf stage (7 days old). The exposure time was 5 days. The receiving cultivar Salome was exposed to: volatiles from

Fairytale (SeF), volatiles from Anakin (SeA), and clean air (Se0).

Electrical penetration graph recording setup

Aphid stylet activities are commonly monitored by an electrical penetration graph (EPG) device to determine different plant tissues where resistance can occur (Tjallingii 2006). We used an EPG system to determine whether the volatile interactions between barley cultivars induce plant resistance affecting aphid feeding behavior. The experimental setup was placed in a Faraday cage for electrical noise isolation. After a 5-day exposure to either Fairytale (SeF), Anakin (SeA), or clean air (Se0), receiving cultivar (Salome) plants were moved from exposing the system to a Faraday cage. The second leaf of the plants was fixed with transparent nylon strips with tape at the ends. Because aphids prefer to settle on the abaxial leaf side, this side was faced up (Pettersson et al. 2017). Adult apterous aphids of *R. padi* were collected from colonies using a marten-hair brush (size 0) and starved in a Petri dish for a period of 30 min. A vacuum on a small hole in a pipet tip was deployed to fix aphids (Schliephake et al. 2013). Water-soluble silver glue was placed on the middle-back of the aphid dorsum to attach a thin gold wire of about 1.5–2.5 cm in length to the aphid (18 microns in diameter). The other end of the gold wire was connected to a copper wire electrode soldered to a brass nail that functioned as an aphid electrode and was inserted into the input of the EPG headstage amplifier. Another electrode with a length of 8 cm was placed in the soil.

An eight-channel Giga-8 DC EPG system was employed. Devices with 1G Ohm were used to monitor the probing and feeding behavior of aphids on the different treatments evaluated (EPG Systems, Wageningen, The Netherlands). A USB analog/digital converter card (DI 710-UL) was used to transfer the EPG signals to a PC computer. The duration of the recording was eight hours. To ensure the proper data remained in limited output signals, the adjustments of voltages on the EPG system were manually attempted for later analysis. The aphids that produced nymphs, died, or left the plant were discarded from the analysis. EPG signals were acquired and analyzed using Stylet + software for Windows (EPG systems).

We split the EPG data collection into two experiments, where the first one compared aphid feeding on SeF versus Se0 and the second one compared aphid feeding on SeA versus Se0. In each experiment, aphids were run on the EPG device for six trials, where in each trial we tested four aphids on treatment and four aphids on control. We tested one trial (8 plants) per day until we obtained 20 replicates for SeF and 19 replicates for Se0 (experiment 1) and 22 replicates for SeA and 23 replicates for Se0 (experiment 2).

Due to limited space (an 8-channel EPG device) for simultaneous observations, we run only one treatment against control at a time, in order to accumulate a sufficient sample size for each comparison in as a short time as possible, which ensures similar conditions for the aphids and the plants used. This sets a major limitation on our study, as we cannot directly compare data for SeF with SeA, but need to imply differences between them via relative comparisons with the control treatment.

Electrical penetration graph waveforms and variables

The “Stylet + a” software (EPG Systems) was used to analyze the data from the Stylet + d program (Tjallingii and Esch 1993). This software defines clear waveform patterns to determine different phases of stylet performance during aphid penetration and feeding. We used online EPG-Calc 6.1.7 software to calculate different EPG variables (Giordanengo 2014). Consequently, waveform data were calculated based on the several sequential and non-sequential variables of standardized EPG-variable listed on epgsystems.eu. Twenty-nine different EPG variables were used to assess the aphid feeding behavior on different treatments in both experiments. Waveforms in certain phases of aphid feeding behavior were selected for analyses, including: none probing (NP), probing (Pr), pathway (C), potential drops (Pd), sieve element salivation (E1), phloem sap ingestion (E2), stylet penetration difficulties (F), and xylem phase (G) (Tjallingii 1990). Waveform “NP” refers to none probing behavior, which is described as no contact or penetration between stylet and plant tissues (studied variables: number of non-probing (n_NP), average of non-probing (a_NP), and duration of non-probing (s_NP)). Waveform “Pr” refers to the general probing activity, during which the stylet penetrates the plant tissues (studied variables: number of probing (n_Pr), average of probing (a_Pr), total duration of probing (s_Pr), number of first brief probes (n_bPr), and time to first probe (t > 1Pr)). Waveform “C” refers to the pathway phase, described as intercellular penetration movements of the stylet (studied variables: number of C (n_C), average of C (a_C), and total duration of C (s_C)). Waveform potential drops “Pd” describe brief intracellular stylet punctures in the stylet pathway (studied variables: number of potential drop (Pd), and total duration of potential drop (s_Pd). Phloem activity consists of two waveforms: E1 and E2. Waveform “E1” refers to sieve element salivation at the beginning of the phloem phase (studied variables: time to first E (t > 1E), number of single E1 (n_sgE1), number of E1 (n_E1), and total duration of E1 (s_E1)). Waveform “E2” refers to phloem sap ingestion with concurrent salivation (studied variables: time to first E2 (t > 1E2), number of E2 (n_E2), total duration of E2 (s_E2), time to first sustained E2 (t > 1sE2), number of sustained E2 (n_sE2), total duration of sustained E2 (s_sE2), number of E12 (n_E12), and total duration of E12 (s_E12)). Waveform “G” refers to active xylem sap/water ingestion activity (studied variables: number of G (n_G), and total duration of G (s_G)). Waveform “F” is the derailed stylet mechanics, indicating stylet penetration difficulties (studied variables: number of times stylet derailed (n_F), and total duration of stylet derailed (s_F).

Aphid relative growth rate

To test aphid growth, 24-h-old aphids were introduced to receiving cultivar (Salome) after the plants had been exposed for 5 days to volatiles of Fairytale, Anakin, or clean air. The observations were carried out in the exposing system where the receivers with aphids were exposed to volatiles from emitters until the end of the experiment. To produce first-instar nymphs, adult apterae of *R. padi* were randomly selected from aphid culture and placed on eight-day-old oat plants for a period of 24 h. The first instars (24-h-old nymphs) were weighted by using the microbalance (Mettler Toledo, USA). One 24-h-old nymph was placed on each receiving plant (Salome). After 5 days, each nymph was re-weighed and the procedure was repeated in several trials, resulting in 16 replicates for the SeF, 18 replicates for SeA, and 22 replicates for Se0. We used aphid weights to calculate the mean relative growth rate based on the equation suggested by Radford (1967):

$$\text{MRGR } (\mu g/\mu g/\text{day}) = \left(\log W_2 - \log W_1\right)/t_2 - t_1$$

where MRGR = mean relative growth rate, W_1 = weight at the first weighing, W_2 = weight at the second weighing, and t_2–t_1 = the time (days) between first (t_1) and second (t_2) weighing.

Aphid development time

The 24-h-old nymphs of *R. padi* were placed between the first leaf and stem of a single Salome plant in the receiving cages. If the nymph disappeared (e.g., due to unsuccessful establishment or mortality), a new 24-h nymph was released the next day. The nymphs were monitored until they produced the first offspring. The day of introducing 24-h-old nymphs on the plant was counted as day 1. The aphid development time was calculated from day 1 to the day of producing the first offspring. The experiment was repeated in several trials until there were 20 replicates for SeF, 18 for SeA, and 19 for Se0. The observations of aphid development and intrinsic rate of increase were carried out in the same way as for the aphid relative growth rate.

Aphid intrinsic rate of increase

After development time observations, we started recording the intrinsic rate of increase (r_m). The day of producing the first offspring was recorded as day 1, and the total number of nymphs produced on each plant was counted after the following 5 days. We obtained 20 replicates for SeF, 18 for SeA, and 19 for Se0. The fecundity of an individual aphid to the intrinsic rate of increase (r_m) was calculated based on Wyatt and White (1977):

$$r_m = \left(\ln M_d x\, c\right)/d$$

where M_d is the number of nymphs produced by the adult in the first d days of reproduction after the adult molt. The constant ($c = 0.738$) is an approximation of the proportion of the total fecundity produced in the first days of reproduction.

Statistical analyses

The statistical analyses were carried out with the R statistical software (R Core Team 2021). Due to the non-normal distributions of most EPG data, the Wilcoxon rank-sum test (unpaired test) was used for the majority of the variables. For the variables that met the assumptions of parametric tests, general linear models (GLM) were employed (package lme4). We used GLM with the Poisson family to analyze count data (e.g., number of probing) and Gamma family to analyze the continuous variables (e.g., time to first sustained E2). The models were validated by graphic examination of residual plots (Zuur et al. 2010) and overdispersion tests in the DHARMa package. The $\alpha = 0.05$ significance level was applied to test the differences between treatments. Twenty-nine variables were analyzed and compared for each experiment (Table 1).

Generalized linear models (GLMER) were used to analyze response variables aphid development time, intrinsic rate of increase, and relative growth rate, by using the Gamma family with a log link. As a fixed factor, we used the cultivar combination with the categories of SeF, SeA, and Se0, and as a random effect we used trial. The control treatment was used as a reference category in the models, but in order to obtain estimates, errors and p values for pairwise comparisons between SeA and SeF, we rerun the models with SeA as the reference category.

Results

Aphid feeding behavior

The different variables used to analyze aphid *R. padi* feeding behavior at different phases are summarized in Table 1. A significant increase in the number of aphid non-probing (Wilcoxon, $p=0.02$) and probing (Wilcoxon, $p=0.01$) was recorded in SeF compared to Se0. It took approximately twice as long for aphids to probe on SeF compared to Se0. The average duration of aphid probing was significantly lower in SeF than in Se0 (GLM, Estimate $= -0.51$, SE $=0.24$, $t=-2.06$, $p=0.04$). No significant differences between SeA and Se0 were detected in non-probing and probing phases (Table 1).

Table 1 Comparisons of feeding behavior variables (means ± SEM) of *Rhopalosiphum padi* on cultivar Salome exposed to Fairytale (SeF) and Salome exposed to clean air (Se0) for experiment 1, and Salome exposed to Anakin (SeA) and Salome exposed to clean air (Se0) for experiment 2. Most of the variables were analyzed by Wilcoxon rank-sum test and the variables with (a) in the table were analyzed by using GLM models to compare the differences between treatments in each experiment with $p \leq 0.05$. Statistical differences between treatments are highlighted in bold and with asterisks

Variables	Experiment 1			Experiment 2		
	SeF	Se0	P	SeA	Se0	P
Number of replicates	20	19		22	23	
None-probing and probing (tissue penetration)						
1. Number of none probing (n_NP)	**6.4 ± 1.03**	**3.42 ± 0.66**	**0.02***	6.77 ± 1.46	6.83 ± 0.92	0.76(a)
2. Average of none probing (a_NP) (min)	10.4 ± 4.66	19.51 ± 9.45	0.71	11.08 ± 2.73	9.34 ± 1.86	0.91
3. Total duration of none probing (s_NP) (min)	54.77 ± 14.21	52.21 ± 22.22	0.47	71.53 ± 17.52	69.44 ± 13.18	0.61
4. Number of probing (n_Pr)	**6.35 ± 1.02**	**3.35 ± 0.64**	**0.01***	6.55 ± 1.47	6.7 ± 0.92	0.68(a)
5. Average of probing (a_Pr) (min)	**134.07 ± 30.17**	**234.31 ± 38.74**	**0.03*(a)**	183.32 ± 39.38	122.44 ± 27.57	0.38(a)
6. Total duration of probing (s_Pr) (min)	425.02 ± 12.22	427.6 ± 22.21	0.47	408.34 ± 17.52	410.44 ± 13.18	0.61
7. Number of first brief probe (< 3 min) (n_bPr)	0.75 ± 0.28	0.79 ± 0.27	0.75	0.59 ± 0.2	0.48 ± 0.19	0.60(a)
8. Time to first probing (t > 1Pr)	15.98 ± 9.42	10.55 ± 4.36	0.98	3.87 ± 1.55	11.96 ± 4.64	0.53
Pathway phase						
9. Number of C	**13.35 ± 1.57**	**7.42 ± 1.21**	**0.007***	13.45 ± 2.28	16.74 ± 1.7	0.12
10. Average of C (min)	7.36 ± 0.46	9.58 ± 1.51	0.54	6.84 ± 0.53	6.61 ± 0.58	0.53
11. Total duration of C (min)	**99.72 ± 12.93**	**56.45 ± 8.15**	**0.03***	86.6 ± 14.33	104.14 ± 11.29	0.22
12. Number of potential drop (Pd)	**73.35 ± 10.75**	**37.11 ± 4.91**	**0.01***	69.05 ± 14	86.83 ± 12.03	0.13
13. Total duration of potential drop (s_Pd) (min)	**5.25 ± 0.73**	**2.7 ± 0.36**	**0.01***	4.97 ± 1.03	6.3 ± 0.83	0.6
Phloem phase						
14. Time to first E (t > 1E)	96.35 ± 15.85	73.27 ± 16.52	0.29(a)	89.79 ± 22.94	108.35 ± 21.2	0.36
15. Number of single E1 (n_sgE1)	0.95 ± 0.36	0.53 ± 0.3	0.15	1.77 ± 0.35	1.83 ± 0.43	0.79
16. Number of E1 (n_E1)	**4.4 ± 0.61**	**3 ± 0.71**	**0.05***	4.18 ± 0.66	4.57 ± 0.66	0.71
17. Total duration of E1 (s_E1) (min)	**3.56 ± 1.49**	**2.1 ± 0.91**	**0.03***	7.14 ± 2.1	11.89 ± 3.1	0.38
18. Time to first E2 (t > 1E2)	125.39 ± 20.14	76.54 ± 16.3	0.09	113.55 ± 28.19	156.89 ± 24.48	0.09
19. Number of E2 (n_E2)	3.45 ± 0.55	2.47 ± 0.6	0.11	2.23 ± 0.34	2.48 ± 0.35	0.62
20. Total duration of E2 (s_E2) (min)	**202.11 ± 35.98**	**314.93 ± 32.32**	**0.05***	227.56 ± 33.64	148.6 ± 25.91	0.09
21. Time to first sustained E2 (t > 1sE2) (min)	**215.04 ± 33.62**	**90.15 ± 17.08**	**0.008***	180.17 ± 36.68	241.6 ± 34.81	0.19
22. Number of sustained E2 (n_sE2)	1.15 ± 0.2	1.21 ± 0.12	0.56	1.41 ± 0.25	1.13 ± 0.16	0.40(a)
23. Total duration of sustained E2 (s_sE2) (min)	**197.01 ± 36.3**	**312.8 ± 32.69**	**0.03***	226.01 ± 33.71	144.87 ± 25.83	0.09
24. Number of E12	3.45 ± 0.55	2.42 ± 0.58	0.09	2.18 ± 0.34	2.39 ± 0.34	0.65
25. Total duration of E12 (min)	**204.25 ± 35.9**	**316.42 ± 32.43**	**0.05***	233.34 ± 34.06	157.66 ± 26.63	0.1
Xylem phase						
26. Number of G (n_G)	**1.55 ± 0.4**	**0.47 ± 0.14**	**0.001*(a)**	**1.14 ± 0.18**	**1.83 ± 0.15**	**0.004***
27. Total duration of G (s_G) (min)	**44.87 ± 11.99**	**11.42 ± 5.49**	**0.01***	**67.03 ± 15.54**	**101.97 ± 12.08**	**0.01***
Stylet penetration difficulty						
28. Number of Stylet derailed (n_F)	1.7 ± 0.3	1.32 ± 0.36	0.33(a)	**1.09 ± 0.35**	**2.17 ± 0.43**	**0.04***
29. Total duration of Stylet derailed (s_F) (min)	74.63 ± 23.31	42.64 ± 19.29	0.09	19.78 ± 6.11	43.42 ± 12.4	0.12

In SeF, the number of aphid attempts in C phase (pathway phase) was significantly higher (Wilcoxon, $p = 0.006$) and aphids spent considerably more time in C phase (Wilcoxon, $p = 0.03$) than in Se0. There was also a significant increase in the number of potential drops (Pd) and total duration of potential drops (Wilcoxon, $p = 0.01$, $p = 0.01$, respectively) in SeF compared to Se0. In contrast, no differences in these variables were detected between SeA and Se0 (Table 1).

In the case of the phloem phases, aphids had a slightly higher number of attempts to salivation phase (E1) (Wilcoxon, $p = 0.05$) with significantly longer duration (E1) in SeF compared to Se0 (Wilcoxon, $p = 0.03$). The total duration of the phloem ingestion phase (E2) was slightly shorter (Wilcoxon, $p = 0.05$), and the total duration of sustained phloem ingestion (sE2) was significantly reduced (Wilcoxon, $p = 0.03$) in SeF compared to Se0. Time to the first sustained phloem ingestion ($t >$ 1sE2) was significantly longer in SeF than Se0 (Wilcoxon, $p = 0.008$). On the other hand, there were no differences in these variables between SeA and Se0 (Table 1).

The number of attempts and the total duration of xylem ingestion (G) were higher in SeF than in Se0 (GLM, Estimate = 1.18, SE = 0.37, $t = 3.13$, $p = 0.001$; Wilcoxon, $p = 0.01$, respectively). Interestingly, these two variables were lower in SeA than Se0 (Wilcoxon, $p = 0.004$, $p = 0.01$, respectively) (Table 1). There was a significantly lower number of stylet derailment (F) (Wilcoxon, $p = 0.04$) but there was no difference in the total time of stylet derailment between SeA and Se0 (Wilcoxon, $p = 0.12$). No differences in these variables were detected between SeF and Se0.

Overall, in experiment 1, SeF aphids spent 48% of the time in non-phloem ingestion phases vs. 32% in Se0, 43% vs. 66% in phloem ingestion and 9% vs. 2% in xylem ingestion, whereas in experiment 2, SeA aphids spent 37% of time in non-phloem ingestion vs. 45% in Se0, 49% vs. 33% in phloem ingestion, and 14% vs. 21% in xylem ingestion.

Aphid growth and development

The relative growth rate of individual *R. padi* nymphs after 5 days ranged from 0.29 to 0.66 µg/day. The GLM analysis showed that the relative growth rate of aphids was significantly reduced in SeF compared to Se0 (GLM, Estimate = − 0.12, SE = 0.05, $t = -2.26$, $p = 0.02$) and SeA (GLM, Estimate = − 0.14, SE = 0.05, $t = -2.45$, $p = 0.01$). However, no significant differences between SeA and Se0 (GLM, Estimate = 0.01, SE = 0.05, $t = 0.30$, $p = 0.76$) were detected (Fig. 1).

The development time of aphids ranged from 5 to 8 days to reach the adult stage and produce the first batch of new offspring. A significant increase was detected in SeF compared to SeA (GLM, Estimate = 0.1, SE = 0.03, $t = 2.81$, $p = 0.004$), while no difference was observed between

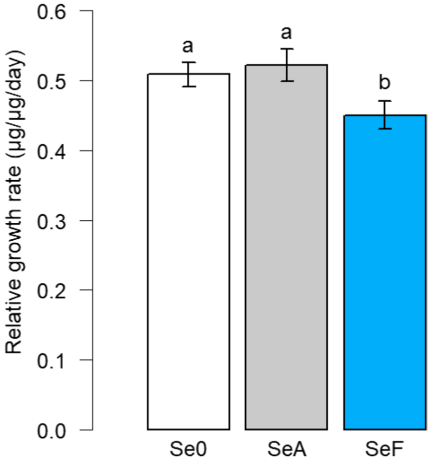

Fig. 1 Mean relative growth rate of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

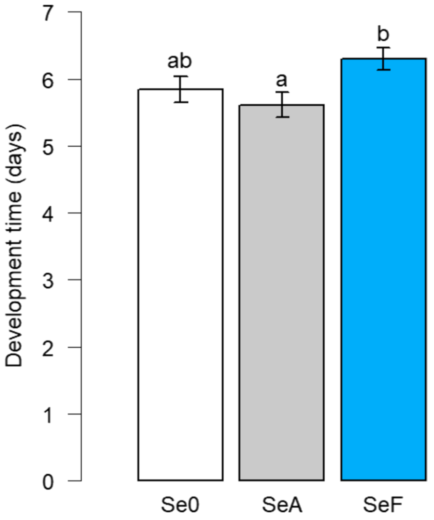

Fig. 2 Mean development time of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

SeF and Se0 (GLM, Estimate = 0.05, SE = 0.04, $t = 1.46$, $p = 0.14$) (Fig. 2).

The potential of aphids to produce new nymphs ranged from 0.47 to 0.64 per day. We observed that aphids on SeF had a significantly lower intrinsic rate compared to on Se0 (GLM, Estimate = − 0.07, SE = 0.02, $t = -2.61$, $p = 0.008$) and SeA (GLM, Estimate = − 0.08, SE = 0.02, $t = -2.99$, $p = 0.002$), respectively. In contrast, aphid intrinsic rate did not significantly differ on SeA and Se0 (GLM, Estimate = 0.007, SE = 0.02, $t = 0.26$, $p = 0.79$) (Fig. 3).

Discussion

This study revealed that volatile interactions between certain undamaged barley cultivars lead to significant ecological effects by interrupting aphid feeding behavior and reducing performance on exposed plants. We have shown that aphid feeding behavior, growth rate, and intrinsic rate were significantly reduced on Salome after exposure to Fairytale, but no differences were found after exposure to Anakin. Our results confirmed the hypothesis that the genetic identity of emitter cultivar expressed through their specific volatile profile can induce resistance factors in the receiving cultivar, which affect aphid performance. A recent field study shows that aphid populations decreased most in the mixture of Salome and Fairytale compared to their pure stands, but not in Salome and Anakin mixture (Dahlin et al. 2018). Under field conditions, several potential mechanisms could contribute to reduced aphid performance (e.g., root interactions, direct competition). However, our findings suggest that volatile interactions between specific undamaged cultivars present one of the underlying mechanisms responsible for disrupting aphid feeding and population development in cultivar mixtures.

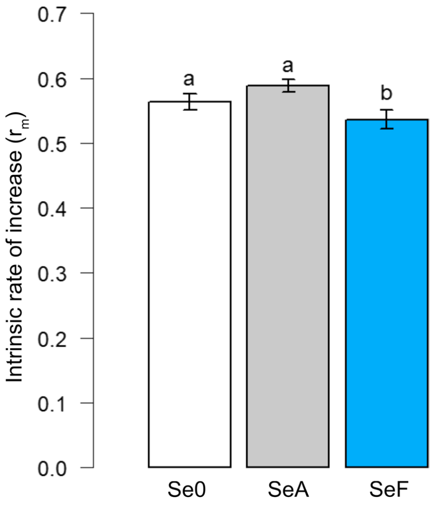

Fig. 3 Mean intrinsic rate of increase (r_m) of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

Both constitutive and induced resistance to aphids can be located in specific plant tissues, and monitoring aphid stylet activity by electrical penetration graph (EPG) technique has been used for the identification of plant tissues where resistance factors against aphids are expressed (Tjallingii 2006). Constitutive resistance factors located in the peripheral layers of the plant tissues make *R. padi* probed slower on resistant than on susceptible genotypes of *Triticum aestivum* L. (Singh et al. 2020). In the present study, volatile interactions between undamaged barley cultivars triggered a similar probing behavior in *R. padi.* Thus, in SeF aphids spent more time in the pathway phase, and longer to reach the first sustained phloem ingestion phase than on Se0, while there was no difference between SeA and Se0. This suggests that only volatiles from specific cultivar could induce resistance factors in epidermis and mesophyll. Volatiles from damaged plants can induce late responses by regulating the primary and secondary metabolism in the receiving plants (Brosset and Blande 2022). The observed negative effects of aphid feeding behavior from mesophyll to phloem on SeF suggest that volatiles from undamaged Fairytale could trigger a response in the receiving plant (Salome) through enhancing resistance factors against aphids. These changes in the aphid feeding behaviors during the pathway could suggest both inter- and intracellular factors. Plant susceptibility to aphids can depend on the morphological characteristics of plant tissues. It has been shown that large intercellular space appearing with a smaller number of mesophyll cells, thinner leaves and thinner guard cells in vascular bundles could make plants more susceptible to aphids (Singh et al. 2020).

Also, changes in plant physiology may affect signaling pathways, expression of defense-related genes, and phloem sap quality (Dinant et al. 2010; Leybourne et al. 2019). These changes could be induced by volatile interactions, and disrupt aphid feeding behavior from epidermis and mesophyll to phloem, resulting in reduced aphid weight and offspring production. Our EPG data showed that volatiles from Fairytale can induce resistance in Salome and interfere in aphid feeding behavior from mesophyll to phloem, and thus reduce aphid performance.

Along the pathway to the phloem, aphid's stylet punctures cells, which is indicated by potential drops (Tjallingii and Esch 1993). In the pre-phloem phase, the number and total duration of potential drops were significantly higher on SeF compared to Se0 (experiment 1), whereas no differences were detected in SeA compared to Se0 (experiment 2). However, the importance of the increased number of potential drops in plant resistance against aphids is still unknown (Sun et al. 2018). Volatiles from damaged plants, such as methyl salicylate, induce effects on the leaf surface resistance factor, prolonging the time until the first probe on exposed, compared to unexposed, barley plants (Ninkovic et al. 2021). The recent review paper suggested that volatile cues can induce early responses by changing the receiving plant surface (Brosset and Blande 2022). Conversely, the EPG data in the current study showed no differences in the duration of non-probing between SeF and Se0 or between SeA and Se0, indicating the absence of induced surface resistance by the volatiles from undamaged plants.

Aphids must overcome host plant defenses associated with phloem to succeed in phloem sap ingestion (Tjallingii 2006). The salivation period (E1) is recognized as the initiation of phloem activities and it is usually followed by the phloem sap ingestion period (E2). Our results showed that the frequency and duration of salivation (E1) were significantly higher in SeF compared to Se0, but not significantly higher in SeA compared to Se0. Several proteins are found in watery saliva, some of which play important roles in biochemical activity and could either function as elicitors or suppressors of plant defense (Goodspeed et al. 2012). For instance, the salivation into the sieve elements during feeding suppresses phloem wound responses causing phloem occlusion, which is considered as a physical barrier preventing blockage of the sieve elements (Pettersson et al. 2017). It is suggested that higher levels of glycerol, trehalose, asparagine, and octopamine play important roles as defensive chemical compounds for phloem resistance (Greenslade et al. 2016). The observed higher frequency of aphid salivation in Salome exposed to Fairytale plants may suggest that receiver plant resistance occurs in phloem through activating defensive chemicals.

Phloem resistance factors could be due to the mechanical blocking of the sieve element after puncturing and the changes in the composition of the phloem sap (Van Helden and Tjallingii 1993), e.g., ratios of phloem sap components (amino acids and sugar) (Will and Van Bel 2006; Dinant et al. 2010), or the presence of certain proteins responsible for phloem sealing (Mutti et al. 2008). The changes in host plant morphology and physiology could also induce phloem-based resistance, by reducing phloem sap ingestion (Guo et al. 2012; Greenslade et al. 2016; Simon et al. 2017). The observed shorter duration in phloem ingestion and sustained phloem ingestion suggest that phloem-based resistance could occur in SeF. Still, it is unknown whether volatile interactions between undamaged plants may induce phloem resistance factors, which may create difficulties for aphids to engage in phloem after salivation and to maintain phloem ingestion.

Aphids may ingest xylem in order to balance the osmotic effects related to a huge amount of phloem sap ingestion (Pompon et al. 2010). Our data revealed that aphids spent a significantly shorter duration in phloem ingestion and a longer duration of xylem ingestion in SeF compared to Se0. This result is in line with a recent study suggesting that aphids increase xylem ingestion due to the reduction in phloem sap ingestion (Escudero-Martinez et al. 2021). In addition, aphid starvation is shown to increase xylem ingestion (Ramírez and Niemeyer 2000). According to these findings, we can speculate that the increase in xylem ingestion could be due to the poor quality of phloem sap, which is also indicated by decreased phloem sap ingestion on SeF.

The development time, fecundity, individual size, life span and reproduction of aphids can be related to the quality of the host plant (Berminghnam and Wikinson 2009; Srisakrapikoop et al. 2021). Our data show that there is a significant reduction in aphid relative growth rate and intrinsic rate in SeF, compared to Se0 and SeA (Figs. 1 and 3). The observed reduction in aphid growth corresponds to the disruption of feeding behavior on SeF, showing that there is a linkage between aphid feeding behavior and performance. It is possible that certain volatiles from Fairytale directly affect Salome as host, which effectively delays aphid feeding behavior and growth. The observed significantly lower number of aphid offspring and weight on SeF could be due to the changes in phloem sap quality, which is also indicated by the shorter duration of phloem ingestion and sustained phloem ingestion. This supports our hypothesis that volatiles from a specific emitter could negatively affect the phloem sap quality and shorten phloem ingestion duration, which consequently reduces aphid weight and offspring production.

The volatile cues from *Geranium macrorrhizum* (Ameline et al. 2002) and the volatiles from *Ocimum basilicum*, marigold and *Tagetes patula*, basil (Dardouri et al. 2020), as companion plants, have been shown to disrupt feeding behavior and reduce the performance of *Myzus persicae* on sweet pepper. In these studies, the negative effects of volatile interactions on aphids could be observed in a certain companion plant, which is similar to the reduction in *R. padi* performance in laboratory tests on certain wheat cultivar mixtures (Cascone et al. 2015; Grettenberger and Tooker 2017). Our results also confirmed the findings of the field study by Dahlin et al. (2018) where aphid population size was significantly reduced in the mixture of Salome and Fairytale, but not in a combination of Salome and Anakin, and pure stands, suggesting that the induced resistance responses in receiving plants are emitter and receiver specific/dependent. This provides good evidence that certain volatiles from specific emitters could potentially directly affect aphid feeding and indirectly influence the phloem sap quality of the receiving plant, which contributes to reducing aphid weight and number of offspring.

Our study showed strong indications that volatile interactions between cultivars of the same crop species affect aphid response and performance through induced changes in their host plants, but these effects are specific to neighboring cultivar identity. Future studies should focus on volatiles-induced physiological changes within plants that are responsible for reduced aphid feeding and performance. An improved understanding of the underlying mechanisms of volatile interactions between cultivars in cultivar mixtures will contribute to the development of the integrated pest management, leading to the development of crop management systems at higher levels of integration.

Author contributions

VN and SK designed the study. SK and DM conducted the experiments. SK, DR, and VN analyzed the data. SK, DM, and VN wrote the manuscript. SK, DM, DR, SI, and VN edited the manuscript. All authors read, contributed to revisions, and approved the manuscript.

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Availability of data and materials The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human participants or large animals performed by any of the authors. No approval of research ethics committees was required to accomplish the goals of this study because this study was conducted with insects and crop plants.

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Cultivar mixtures provide sustainable pest and disease control, enhancing yield. This thesis demonstrates how cultivars' growth rates vary due to aboveground chemical interactions. Volatile interactions among undamaged plants of cultivars induce increased emissions of specific volatiles in neighbouring plants, affecting aphids. These findings emphasize the significant role of volatiles in both plant-plant and insect-plant interactions, serving as an underlying mechanism for pest control.

Sokha Kheam received his PhD education from the Department of Ecology, SLU, Uppsala, Sweden. He obtained his Master of Science in Teaching from De La Salle University, the Philippines, and his Bachelor of Science in Biology from Royal University of Phnom Penh, Cambodia.

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