17th International Symposium on Insect-Plant Relationships

17th SIP - Abstract Book

Leiden University, Leiden, The Netherlands

25-30 July 2021

Organizing and scientific commitees

Organizing commitee:

Klaas Vrieling - Leiden university

Peter klinkhamer - Leiden university

Scientific commitee:

David Giron - Université de Tours, Tours

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Erik Poelman - Wageningen university & research, Wageningen

Nicole van Dam - German centre for integrative biodiversity research, Leipzig

Table of contents

Program at a glance

Program online Symposium on Insect Plant relationships 2021, SIP17, Leiden, The Netherlands

Central European summertime

UTC+2	**Sunday 25 July**	**Monday 26 July**		**Tuesday 27 July**	**Wednesday 28 July**	**Thursday 29 July**	**Friday 30 July**
10h55-11h00		Announcements		Announcements	Announcements	Announcements	Announcements
11h00-11h45		**Ecology** Keynote **Nicole van Dam**		**Metabolomics** Keynote **Caroline Mueller**	**Multitrophic interactions** Keynote **Consuelo De Moraes**	**Phylogenetics and Evolution** Keynote **Niklas Janz**	**Innovations** Keynote **Matthias Erb**
11h45-12h00		Coffee break		Coffee break	Coffee break	Coffee break	Coffee break
12h00-12h15		Menacer		Xu	Volf	Proffit	Turlings
12h15-12h30		Mertens		Feistel	Bourne	Lin TT	Meijer
12h30-12h45		Pashalidou		Escobar Bravo	Ramirez Serrano	Nathan	Brosset
12h45-13h00		Biere		Hougaard	van Dijk	Dermauw	Waterman
13h00-13h30		Lunch/time to meet		Lunch/time to meet	Lunch/time to meet	Lunch/time to meet	Lunch/time to meet
13h30-14h05		Discussion Topics Lange	Discussion Topics Saha S	Posters session 1	Posters session 1	Posters session 1	Posters session 1
14h05-14h15	Dry run	Lunch/time to meet		Lunch/time to meet	Lunch/time to meet	Lunch/time to meet	Lunch/time to meet
14h15-14h30	**Opening**						
14h30-15h15	**Plenary keynote** **Andre Kessler**	**Genomics** Keynote **Heiko Vogel**		**Insects and weeds** Keynote **Ruth Hufbauer**	**Community ecology** Keynote **John Vandermeer**	**Plant-pollinator interactions** Keynote **Geraldine Wright**	**Closing session** Keynote **Marcel Dicke**
15h15-15h30		Coffee break		Coffee break	Coffee break	Coffee break	Farewell
15h30-15h45		Lin YH		Martin Camargo	Thompson	Tzin	Meeting for next SIP
15h45-16h00		Heckel		Minuti	Costaz	Deconninck	
16h00-16h15		Kloth		Neidel	Kheam	Haverkamp	
16h15-16h30		Steward		van der Zande	Vazquez Gonzales	Querejeta	
16h30-17h00		Tme to meet		Tme to meet	Tme to meet	Tme to meet	

Colors indicate elements belonging together and are introduced by a keynote

Poster session 1	**Poster session 2**	**Poster session 3**	**Poster session 4**
Bellec	Boutsi	Borg	Beretta
Biru	Leandro	Bovio	Cuny
Cibils Stewart	Dort	Boyer	Koupilová
Cofer	Douma	Bühler	Mannall
Croijmans	Hernandez Gutierrez	Chia	Minchev Ivanov
de Lange	Karssemeijer	Grunseich	Putra
Dorland	Lange	HAO	Rodriguez-Saona
Fontanilla	Leclerc	Johnson	Rusman
Gaytan	Saha S	Kamps	Suijkerbuijk
Islam	Tadmor	Sadeh	Voß
Legarrea	Vitiello	Schimmel	Wilberts
Martín Cacheda	Wang	Wahyuni	
Paulmann	Wintraube	Galman	
Quijano	Saha H	Kulkarni	
Ruhanen	Chiriboga Morales	Touw	

Program SIP 2021

Sunday 25 July 2021

Welcome

14h15 Peter Klinkhamer and Klaas Vrieling

Opening lecture

Chair : Peter Klinkhamer

14h30 **André Kessler: Why plants communicate**
15h15 Closure of the day

Program schedule

Monday 26 July

10.55 Announcements

Ecology of plant-insect Interactions

Chair: Caroline Mueller

Keynote

11h00 **Nicole van Dam: Plant responses to aboveground and belowground herbivores: Fighting a war on two fronts**

11h45 Coffee break

Orals

12h00 Kathleen Menacer: Challenging the Preference-Performance Hypothesis in an above-belowground insect

12h15 Daan Mertens: Predicting the outcome of plant-mediated herbivore interactions from prevalence observations in the field.

12h30 Fonteini Pashalidou: Plant volatiles induced by herbivore eggs prime defences and mediate shifts in the reproductive strategy of receiving plants

12h45 Arjen Biere: How root fungi interfere with plant herbivore resistance: insights from a transcriptome study in *Plantago lanceolata*.

13h00 Time to meet/Lunch

Discusion topics (parallel sessions)

13h30 Claudia Lange: Implications of switching invertebrate microbiomes on interactions with plants

13h30 Surya Saha: AgriVectors: a community resource for arthropod vectors and agricultural pathosystems

14h05 Time to meet/Break

Genomics of plant-insect interactions

Chair: Andre Kessler

Keynote

14h30 **Heiko Vogel: A genomic perspective on insect adaptive strategies to host plant defenses**

15h15 Coffee break

Orals

15h30 Yu-Hsien Lin: Spit it out: Characterization of an insect salivary enzyme that impacts green leaf volatile emission in plants

15h45 David Heckel: Genomic analysis of a partial host shift: the crucifer specialist *Plutella xylostella* on pea

16h00 Karen Kloth: One for all? Broad-spectrum plant resistance to aphids and whiteflies

16h15 Rachel Steward: Tissue-specific patterns of alternatively spliced transcripts hallmark the evolutionary history of plant usage by *Vanessa cardui*

16h30 – 17h00 Time to meet/end of day

Tuesday 27 July

10.55 Announcements

Metabolomics of plant-insect interactions

Chair: Nicole van Dam

Keynote

11h00 **Caroline Mueller : Insights from ecometabolomics into intraspecific chemodiversity of plants and interactions with insects**

11h45 Coffee break

Orals

12h00 Wei Xu: Electroantennogram responses of the parasitoid wasp, *Diadegma semiclausum*, to plant-related odours

12h15 Felix Feistel: The role of toxic quinolizidine alkaloids from *Genista* plants (Fabaceae) in aphid-plant interactions

12h30 Rocio Escobar Bravo: Plant-mediated facilitation of leafminer metamorphosis in the soil

12h45 Katia Hougaard: Race-specific and R-gene-dependent transcriptome responses to pea aphid infestation in *Medicago truncatula*

13h00 Time to meet/Lunch

13 h30 **Poster session 1**

14h05 Time to meet/Break

Insects and weed control

Chair: Klaas Vrieling

Keynote

14h30 **Ruth Hufbauer : Plant-insect interactions in the context of biological invasions**

15h15 Coffee break

Orals

15h30 Ana Martin Camargo: Preference and performance of the biocontrol agent *Aphalara itadori* on invasive knotweed species in the Netherlands

15h45 Gianmarco Minuti: Climatic suitability of the invasive aquatic Iris pseudacorus L. and its candidate biocontrol agent *Aphthona nonstriata* (Goeze) in the Southern Hemisphere

16h00 Veronika Neidel: The risk of being eaten: prey metabarcoding reveals which seed traits drive carabid weed seed predation in European wheat fields

16h15 Els van der Zande: Biological control in circular agriculture - Using insect side-streams to increase crop growth and parasitoid recruitment

16h30 – 17h00 Time to meet/end of day

Program schedule

Wednesday 28 July

10.55 Announcements

Multitrophic interactions

Chair: Matthias Erb

Keynote

11h00 **Consuelo De Moraes: Chemical communication in inter-specific ecological interactions**

11h45 Coffee break

Orals

12h00 Martin Volf: A mosaic of induced and non-induced branches promotes variation in plant traits, predation and leaf palatability to insect herbivores

12h15 Mitchel Bourne: The effect of parasitized aphids on plant-mediated interaction networks

12h30 Beatriz Ramirez Serrano: Influence of mycorrhizal symbiosis and fertilization on the performance of *Spodoptera exigua*: are effects mediated by a change of the insect gut microbiota?

12h45 Laura van Dijk: Direct and insect-mediated effects of pathogens on plant growth and fitness

13h00 Time to meet/Lunch

13 h30 **Poster session 2**

14h05 Time to meet/Break

Community ecology

Chair: Geraldine Wright

Keynote

14h30 **John Vandermeer: The Community Ecology of Herbivore Regulation in an Agroecosystem: Lessons from Complex Systems**

15h15 Coffee break

Orals

15h30 Morgan Thompson: Systemic plant responses to belowground herbivory deter aboveground herbivores

15h45 Thibault Costaz: Parasitoids response to extreme temperature events are driven by the structure of their surrounding community.

16h00 Sokha Kheam: Volatile interaction between different barley cultivars affects aphid feeding behaviour

16h15 Carla Vázquez Gonzales: Tree species composition and water availability influence direct and indirect defences in *Quercus ilex*

16h30 – 17h00 Time to meet/end of day

Thursday 29 July

10.55 Announcements

Phylogenetics and Evolution

Chair: Consuelo De Moraes

Keynote

11h00 **Niklas Janz: The Parasite Paradox: host repertoires and the diversification of butterflies**

11h45 Coffee break

Orals

12h00 Magali Proffit: Phylogenetic constraints on the evolution of floral scents in a nursery pollination mutualism

12h15 Tiantian Lin: Evolutionary changes in an invasive plant support the defensive role of plant volatiles

12h30 Pooja Nathan: Mutualism promotes range expansion in both plant and insect partners

12h45 Wannes Dermauw: Extreme gene and intron loss in the miniature genome of the tomato russet mite

13h00 Time to meet/Lunch

13 h30 **Poster session 3**

14h05 Time to meet/Break

Plant-pollinator interactions

Chair: Marcel Dicke

Keynote

14h30 **Geraldine Wright : The sweet taste of nectar: novel mechanisms for taste coding revealed in bees**

Summary

15h15 Coffee break

Orals

15h30 Vered Tzin: Desert time: Phylogeny and abiotic conditions shape the diel floral emission patterns of desert Brassicaceae species

15h45 Gwenaëlle Deconninck: Specificity of pollinators between syntopic species of the genus *Ceropegia* (Apocynaceae) sharing the same pollination syndrome

16h00 Alex Haverkamp: Developing a taste for sweets: Sugar detection and preference in *Manduca sexta* caterpillars and adults reflects leaf and nectar feeding lifestyles

16h15 Marina Querejeta: Pollination by wild bees in sunflower crops: a metabarcoding approach

16h30 – 17h00 Time to meet/end of day

Program schedule

Friday 30 July

10.55 Announcements

Innovations

Chair: Niklas Janz

Keynote

11h00 **Matthias Erb: How multifunctional secondary metabolites shape plant-herbivore interactions**

11h45 Coffee break

Orals

12h00 Ted Turlings: A horizontal gene transfer event has empowered whiteflies with the ability to neutralize common plant defense compounds

12h15 Davy Meijer: The effects of red to far-red light ratio on plant-insect interactions across trophic levels

12h30 Agnes Brosset: Exposure to (Z)-11-hexadecenal increases *Brassica nigra* susceptibility to subsequent herbivory.

12h45 Jamie Waterman: Short-term exposure to silicon rapidly enhances plant resistance to insect herbivory

13h00 Time to meet/Lunch

13 h30 **Poster session 4**

14h05 Time to meet/Break

Closing Session

Chair: Peter Klinkhamer

Keynote

14h30 **Marcel Dicke: Microbial involvement in insect-plant interactions**

15h15 Farewell

15h30 Meeting

Why plants communicate

André Kessler

Cornell University, Department of Ecology & Evolutionary Biology, Ithaca, United States

All living organisms are exposed to ever-changing biotic and abiotic environments with varying degrees of predictability. While mobile organisms can just move to a more favorable part of their habitat, sessile organisms, such as most plants, have to rely mostly on adjustments in growth and metabolism to cope with changes in their environment, especially those associated with interacting arthropods. Plant interactions with the arthropod community are largely mediated by a chemical language that we try to decipher and understand, functionally, as a major component of plant behavioral biology. Great work has been done to understand direct and indirect defensive as well as reproductive functions of many plant secondary metabolites. However, that work has exposed some new interesting questions that address both, the evolutionary implications as well as the applicability of the plants' abilities to transmit chemical information. Those include: Why are there so many plant secondary metabolites that seemingly do the same (functional redundancy) while a single compound can have multiple metabolic and ecological functions? How does natural selection by many interactors seemingly using the same chemical language shape the evolution of chemical cues and signals? How is chemical information processed in the quackmire of chemical cues emitted from diverse plant communities (e.g. associational effects, environmentally acquired traits)? This presentation will try to reflect on some of those aspects while focusing on tall goldenrod, Solidago altissima, and its interactors as ecological model system to understand plant communication and behavior. A serious of micro-evolutionary studies and functional analyses will be reviewed to address the factors that drive the evolution of plant induced defenses and plant-to-plant information transfer. Moreover, soil-microbial communities and soil contaminations with toxic metals will be discussed as environmentally-acquired plant resistance that affects the evolution of plant endogenous resistance.

Plant responses to aboveground and belowground herbivores: Fighting a war on two fronts

Nicole M. van Dam[1,2], Axel Touw[1,2], Tomonori Tsunoda[3]

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Plants are attacked by multiple herbivores, both above and below the ground. Producing chemical defences can be costly; therefore, we hypothesized that plants optimize defence production and allocation. The Optimal Defense Theory (ODT) predicts that organs with the highest value to plant fitness as well as those with the highest risk to receive herbivore damage, have the highest levels of defences. Until recently, the ODT was tested mostly for aboveground plant organs. Our research takes the ODT belowground, in particular to taproots, lateral roots and fine roots. We use glucosinolate (GSL)-containing plants species to study constitutive and herbivore-induced GSLs and analyse the molecular mechanisms underlying allocation patterns. Core structural organs, such as taproots and stems, contained the highest levels of constitutive GSL levels. Generalist beetle larvae preferred feeding on fine roots, whereas larvae of the specialist herbivore Delia radicum preferred taproots. Herbivory on different root classes always elicited the strongest GSL increase in the taproots, independent of which root class was damaged by herbivory. We performed qPCR analyses of specific GSL biosynthesis and GSL transporter genes. The expression of biosynthesis and transporter genes was strongly upregulated in taproot tissues within 12 h after the onset of herbivory. Additionally, we found that structurally related belowground defence compounds, in particular the phytoalexin cyclobrassinin, was induced by D. radicum feeding as well. This suggests that belowground herbivory also elicits anti-microbial defences. Our research adds to a better understanding of how plants optimize defence allocation to the multitude of belowground enemies they face.

Challenging the Preference-Performance Hypothesis in an above-belowground insect

Kathleen Menacer, Anne Marie Cortesero and Maxime R. Hervé

IGEPP-UMR 1349, INRAE, Institut Agro, Univ Rennes 1, 35000 Rennes, France

In the study of host plant selection by phytophagous insects, the Preference-Performance Hypothesis (PPH) is one of the main hypotheses proposed to explain a positive correlation between female oviposition preference and offspring performance. The PPH states that females should preferentially lay eggs on plants providing the best larval development. It has been extensively tested on aboveground insects but its application to species with belowground larvae is still mostly unknown. This study proposes to challenge the PPH in an above-belowground phytophagous insect.

The study was performed on the cabbage root fly (*Delia radicum*), three brassicaceous species (*Brassica oleracea*, *Brassica rapa* and *Sinapis alba*) and four cultivars of *B. rapa* and *S. alba*. Female preference was investigated in inter and intraspecific multiple-choice oviposition tests. In addition, a development experiment was performed with larval performance estimated through survival and life history traits of emerging adults.

Oviposition contrasts were observed showing that *D. radicum* females have both intra and interspecific preferences. Larval survival and performance differed between but not always within species. The PPH was validated at the intraspecific scale but only in *B. rapa* and for some, but not all, of the life history traits. No support for the PPH was found in *S. alba* while results were more ambiguous at the interspecific scale.

This work pleads for a full integration of these insects in the preference-performance debate. Moreover, it raises the importance of measuring several variables to estimate larval performance, and to test the PPH both at the intraspecific and interspecific scales before drawing general conclusions. Further studies with wild hosts would help to determine whether the behavior observed in *D. radicum* females is adaptive in some way, or is a consequence of an uncoupling of chemical signals used by females and determinants of larval performance during the plant domestication process.

Keywords: plant-insect interactions; host plant selection; *Delia radicum*; *Brassicaceae*

Predicting the outcome of plant-mediated herbivore interactions from prevalence observations in the field

Daan Mertens[1*], Maite Fernández de Bobadilla[1*], Quint Rusman[1], Janneke Bloem[1], Jacob C. Douma[2] & Erik H. Poelman

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[*]Equal contributions

Objective

To cope with a diverse community of insect herbivores while minimising metabolic costs to acquire resistance, plants rely on a wide range of inducible defences. The current consensus is that plant responses to initial herbivore attack strongly determine the potential to respond to subsequent herbivory. This may result in compromises in plant resistance to sequential attack by herbivores of different feeding guilds. However, this pattern is found to be riddled with specificity and the ecological outcome of plant-mediated interactions remains hard to predict.

An overlooked but alternative explanation for the physiological hypothesis in predicting plant-mediated interactions is that interactions between plants and the associated herbivore community are not random. Rather, the likelihood of attack is determined by processes structuring the composition and the assembly of herbivore communities. We hypothesise that plants are under selection to incorporate this structure in their defence strategies, i.e. which fitness-impacting herbivores are likely to attack, and when will attack take place.

Method

We performed a greenhouse experiment evaluating the outcome of pairwise interactions between 10 herbivore species mediated by the annual plant *Brassica nigra*. To interpret these results in an ecological framework, we related the outcome of pairwise interactions to the observed prevalence of herbivore species that arrive on induced plants in three years of field observations.

Results

Resistance to a later arriving herbivore species is not explained by feeding guild of the initial attacker. Instead, plants maintained resistance to prevalent herbivores in common patterns of herbivore arrival and compromises in resistance especially occurred for rare patterns of herbivore attack.

Conclusion

Brassica nigra tailors its induced defence strategies to deal with common patterns of sequential herbivore attack and is not compromised in responses to the most prevalent subsequent herbivore attacks. We caution that the basal physiology of plant responses should not be extrapolated to predict the ecological outcomes of herbivore interactions.

Keywords
Plant-mediated interactions, induced plant defence, insect herbivore community, anticipatory strategies.

Plant volatiles induced by herbivore eggs prime defences and mediate shifts in the reproductive strategy of receiving plants

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Plants can detect cues associated with the risk of future herbivory and modify defence phenotypes accordingly; however, our current understanding is limited both with respect to the range of early warning cues to which plants respond and the nature of the responses. Here we report that exposure to volatile emissions from plant tissues infested with herbivore eggs promotes stronger defence responses to subsequent herbivory in two *Brassica* species. Furthermore, exposure to these volatile cues elicited an apparent shift from growth to reproduction in *Brassica nigra*, with exposed plants exhibiting increased flower and seed production, but reduced leaf production, relative to unexposed controls. Our results thus document plant defence priming in response to a novel environmental cue, oviposition-induced plant volatiles, while also showing that plant responses to early warning cues can include changes in both defence and life-history traits.

ABSTRACT TITLE

How root fungi interfere with plant herbivore resistance: insights from a transcriptome study in *Plantago lanceolata*.

AFFILIATIONS

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ABSTRACT TEXT

Objectives – Beneficial microbes in the rhizosphere and roots of plants are increasingly recognized for their potential to prime plants for enhanced defense against leaf chewing herbivores. Mycorrhiza-induced Resistance (MiR) refers to the ability of arbuscular mycorrhizal (AM) fungi to sensitize the plant's immune system, resulting in a faster or stronger activation of jasmonic acid (JA)- signalled defences upon herbivory. However, recent studies have shown a wide range of outcomes in the effect of AM fungi on plant defense against herbivory, suggesting that either MiR is not a universal response, or that other processes induced by AM fungi negate the effects of mycorrhizal priming of defense. Our objective was to gain understanding of how AM fungi affect a broad range of processes involved in plant-herbivore interactions.

Methods – We performed a greenhouse experiment with *Plantago lanceolata* plants subjected to a factorial combination of two treatments: microbial inoculation (inoculation with the AM fungus *Funneliformis mosseae* or mock) and herbivory (8 days of leaf herbivory by the lepidopteran *Mamestra brassica* or undamaged controls). Leaf and root samples were processed for RNA sequencing using an Illumina platform to analyse effects on plant gene expression.

Results – The AM fungus enhanced transcription of genes involved in osmotic and oxidative stress tolerance, but did not prime plants for JA-signalled defense. On the contrary, the AM fungus suppressed induction of terpenoid compounds involved in direct and indirect defense. Furthermore, the fungus suppressed storage of carbon in shoots in the form of starch and oligosaccharides and nitrogen in the form of storage proteins in response to herbivory.

Conclusions - Although priming of herbivore defense by root-associated benefical microorganisms is a well-accepted paradigm, this response is not universal, and microbes have multifaceted effects on the plant's responses to herbivory, contributing to their broad range of effects on plant defense against herbivory.

KEYWORDS
Plant-microbe-insect interactions, priming, AMF, Mamestra

Spit it out: Characterization of an insect salivary enzyme that impacts green leaf volatile emission in plants

Yu-Hsien Lin[1*], Juliette Silven[1], Nicky Wybouw[2], Richard A. Fandino[3], Yueh-Lung Wu[4], Silke Allmann[1*]

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Plants respond to insect feeding with the release of volatiles. These volatiles can have direct toxic effects on the attackers, and they also serve as signalling compounds for the plant itself and for organisms in its nearby environment. Green leaf volatiles (GLVs) are a group of short-chain oxylipins that are readily released from plants upon damage. While it was long thought that the emission of GLVs is simply a consequence of the breakdown of lipid membranes from damaged cells, evidence has gradually accumulated those plants are able to adjust the amount of GLVs released as well as their composition, thereby creating specificity in the volatile signal. However, insects also seem to be able to manipulate this volatile signal for their own benefit. In previous research we have shown that *Manduca sexta* causes the re-arrangement of the GLV *Z*-3-hexenal to *E*-2-hexenal while feeding from wild tobacco plants. However, this change in the volatile signal, which is caused by a salivary enzyme introduced into leaf wounds during feeding, seems to have both, beneficial and detrimental effects for the insect. We thus set out to identify the enzyme responsible for this conversion, to understand its main function for the insect itself and its role in plant-insect interactions. Through biochemical fractionation we identified a salivary enzyme, hexenal isomerase (Hi), that showed clear isomerase activity when ectopically expressed in Arabidopsis and *Nicotiana benthamiana*. Oral secretions of larvae that were mutated in the Hi through CRISPR/Cas9 had lost the activity to convert *Z*-3- into *E*-2-hexenal. Interestingly, these mutants showed clear morphological deficiencies when fed on artificial diet, indicating an important role of this enzyme in development processes. Future research will not only reveal the main role of this enzyme for *M. sexta*, but will also shed light on the phylogeny of Hi in other lepidopteran species.

Keywords: Green leaf volatiles, hexenal isomerase, *Manduca sexta*, oral secretion, CRISPR/Cas9

Genomic analysis of a partial host shift: the crucifer specialist *Plutella xylostella* on pea

David G. Heckel, Max Planck Institute for Chemical Ecology, Jena, Germany
Christopher Ward, University of Adelaide, Adelaide, Australia
Simon W. Baxter, University of Melbourne, Melbourne, Australia

Objective

A population of *Plutella xylostella* (diamondback moth, DBM) was collected from sugar pea in Kenya, and completes its life cycle on pea in the laboratory. This is a partial host shift because although larvae can develop on pea, adult females show no oviposition preference for pea. We sought to determine the genetic basis of this adaptation to a new host.

Method

A genome sequence for the crucifer-adapted strain DBM-W from interspecific hybrids with *Plutella australiana* used PacBio and Illumina sequencing and trio binning. We crossed and intercrossed the pea-adapted strain DBM-P to DBM-W. A QTL analysis of survivorship on pea used SNP markers from Illumina sequencing of progeny. We obtained transcriptome information on larval midgut and head capsule by RNAseq.

Results

When families were split with half reared on kale and half on pea, we detected seven QTLs; some with evidence for pea adaptation and others suggesting a fitness cost or tradeoff to pea-adaptation. Transcript differences were 1) strain differences, 2) primed responses to the host plant or 3) dynamic responses to host plant. Enriched Gene Ontology (GO) terms included 1) hexose and organic substrate catabolic processes, 2) translation and amide biosynthetic processes, and 3) sulfur metabolic processes and non-coding RNA processing. Phase I and Phase II detoxification genes were diffentially expressed in midgut, and chemosensory genes were differentially expressed in head tissue.

Conclusion

DBM larval adaptation to pea is multifactorial and involves changes in gene regulation. Phenotypic plasticity may contribute but probably was more important in the initial colonization of pea in the field, because after >300 generations of rearing exclusively on pea in the laboratory, females still show no evidence of a genetically-encoded preference for pea. Supported by the Grains Research and Development Corporation (Grant 9175870), the Australian Research Council (Grant FT140101303), and by the Max-Planck-Gesellschaft.

KEYWORDS
crucifer specialist, Brassicales, Plutella xylostella, Pisum sativum

One for all? Broad-spectrum plant resistance to aphids and whiteflies

Karen J. Kloth[1], Parth Shah[1], Colette Broekgaarden[2], Cecilia Ström[3], Benedicte R. Albrectsen[3], Marcel Dicke[1]

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Resistance (*R)* genes usually compete in a coevolutionary arms race with reciprocal effectors to confer strain-specific resistance to pathogens or herbivorous insects. We investigated the specificity of *SLI1*, a recently identified *R* gene in Arabidopsis that encodes a small heat shock-like protein involved in resistance to *Myzus persicae* aphids. In a panel with several aphid and whitefly species, SLI1 compromised reproductive rates of three species: the tobacco aphid *M. persicae nicotianae*, the cabbage aphid *Brevicoryne brassicae* and the cabbage whitefly *Aleyrodes proletella*. Electrical penetration graph recording of aphid behaviour, revealed shorter salivations and a 3-to-5-fold increase in phloem feeding on *sli1* loss-of-function plants. The mustard aphid *Lipaphis erysimi* and *Bemisia tabaci* whitefly were not affected by SLI1. Unlike the other two aphid species, *L. erysimi* exhibited repetitive salivations preceding successful phloem feeding, indicating a role of salivary effectors in overcoming SLI1-mediated resistance. Microscopic characterization showed that SLI1 proteins localize in the sieve tubes of virtually all above- and below-ground tissues and co-localize with the aphid stylet tip after penetration of the sieve element plasma membrane. These observations reveal an unconventional *R* gene that escapes the paradigm of strain specificity and confers a broad-spectrum quantitative resistance to phloem-feeding insects. The underlying resistance mechanism is yet to be elucidated, but several phloem-located mechanisms, including protein coagulations and calcium signalling, are hypothesized.

Keywords: Plant resistance, aphids, whiteflies, phloem, *R* genes

ABSTRACT TITLE

Tissue-specific patterns of alternatively spliced transcripts hallmark the evolutionary history of plant usage by *Vanessa cardui*

AFFILIATIONS

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ABSTRACT TEXT

Objective

Most herbivorous insects are specialists, but some, like the butterfly *Vanessa cardui* that feeds on over 60 plant genera, are spectacular generalists. Despite extensive theoretical and empirical research into *why* some insects are generalists, there is still a limited understanding of *how* insects are generalists. Growing evidence highlights the importance of transcriptional regulation to generalization, but other mechanisms of transcriptional variation, such or alternative splicing have been largely unexplored. Here, we compare splicing in *V. cardui* larvae reared on different host plants and evaluated whether patterns reflect the phylogenetic relatedness of hosts or the historical associations between these butterflies and their host plants.

Method

We used RNAseq data from *V. cardui* to quantify differential exon expression patterns in four different tissues from larvae reared on six host plants. These host plants differed both in phylogenetic relatedness and in their history of use as hosts, with three belonging to orders that are used by other members of the *Vanessa* genus (core) and three used exclusively by *V. cardui* (extended).

Results

Patterns of splicing reflected the evolutionary history of host associations rather than the host phylogeny. Surprisingly, differential splicing between core and extend host plants was most prevalent in the fat body. Many of the genes that were differentially spliced between core and extended host plants have previously been shown to have detoxification or metabolic functions, and differentially spliced genes were also enriched for regulatory functions.

Conclusion

Alternative splicing introduces a unique axis of transcriptional variation that is consistently overlooked in studies that focus on whole gene expression. Our results confirm that the molecular mechanisms that enable *V. cardui* to be a generalist do not depend on host phylogeny, but rather a history of use by *Vanessa* butterflies.

KEYWORDS

Alternative splicing, differential expression, generalist, *Vanessa cardui*

Insights from ecometabolomics into intraspecific chemodiversity of plants and interactions with insects

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The use of ecometabolomics has drastically increased in studies of plant-insect relationships. Among other applications, this approach can offer valuable insights into the variation in chemical composition existing among and within species, but also help to disentangle how herbivores deal with such variation in plant chemistry. The importance of variation in chemical phenotypes of organisms for shaping species interactions has often been neglected, although chemical communication is the most ancient mediator of information exchange among individuals. From the plant perspective, intraspecific plant chemodiversity can play an important role for the specific outcome of plant-herbivore interactions. Moreover, such diversity may play a crucial, yet underestimated role in plant invasions. From the insect perspective, metabolomics approaches can help to determine which plant compounds are causing deterrent effects on herbivores and how certain plant toxins may be metabolised after ingestion. The various aspects will be highlighted by presenting several examples. Future research is needed to disentangle the various ecological consequences of chemodiversity and how it is genetically determined and maintained.

Electroantennogram responses of the parasitoid wasp, *Diadegma semiclausum*, to plant-related odours

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Abstract

An increase in insecticide resistance have been reported broadly in the diamondback moth (DBM) (*Plutella xylostella*), promoting interest in the biological control using natural enemies. Parasitoid wasps have long been used as biocontrol agents of DBM, which are hypothesized to use blends of volatiles released from attacked plants to localize hosts. To identify and analyse these compounds and their specificity are the first key step to understand the mechanisms how natural enemies localize host insects within crops.

In this study, canola seedlings were used as the host plant, which was consumed by *P. xylostella* to identify the volatile compounds by using gas chromatography–mass spectrometry (GC-MS). *Diadegma semiclausum* was studied as the DBM parasitoid wasp to examine its antennal responses to various volatile compounds. Scanning electron microscope (SEM) was used to investigate the olfactory sensilla of the male and female *D. semiclausum*.

The results identified eight compounds that were of significant changes in their amounts from DBM-infested canola. Electroantennogram (EAG) results demonstrated a group of physiologically active compounds which can elicit antennal responses of male or female *D. semiclausum*. These compounds might be candidate attractants for *D. semiclausum* to localize the DBM and DBM-damaged plants. Interestingly, male and female *D. semiclausum* showed different responses to certain tested compounds in EAG. SEM results revealed seven types of olfactory sensilla from both *D. semiclausum* male and female adult antennae.

This study identified candidate attractant compounds for *D. semiclausum* and improves our understanding of their olfactory systems, which will help optimize our biological control strategies to control *P. xylostella* in future.

Keywords: Insect olfaction, Diamondback moth, olfactory sensilla, attractant

ABSTRACT TITLE

The role of toxic quinolizidine alkaloids from *Genista* plants (Fabaceae) in aphid-plant interactions

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ABSTRACT TEXT

Objective

The pea aphid (*Acyrthosiphon pisum*), native to legume plants, comprises at least 15 genetically different biotypes, each adapted to just one or a very few different host plants. Only on its respective host plant, an aphid biotype can perform well. One of these biotypes is specialized on *Genista* plants, known to contain quinolizidine alkaloids (QAs). These compounds stimulate and paralyze the central nervous system of animals and thus can ultimately lead to their death. Hence, the question arises whether QAs are responsible for the death of non-adapted biotypes.

Method

To answer this, we first identified the most abundant QAs of *Genista tinctoria* using NMR spectroscopy and high-resolution mass spectrometry. Second we investigated whether the aphids get exposed to these QAs, by evaluating the compounds' distribution within the aphid-plant system. The identified QAs were examined in *G. tinctoria* phloem sap (the aphids' food), different plant parts and organs, as well as in *Genista* biotype aphid tissue and honeydew by LC-MS/MS, MALDI-MS, microscopy and histochemical staining. Finally the toxicity of some of these compounds was studied by adding them into aphid diet.

Results

Eight QAs were found in *G. tinctoria* extracts, one of which being new for *Genista* species and one previously unknown. The QAs showed an organ- and cell-type-specific distribution. Only three of these compounds were found within *G. tinctoria* phloem sap. These three have also been

observed in aphids, their hemolymph, as well as in their honeydew. Some QAs are highly toxic, but only to non-adapted pea aphid biotypes.

Conclusion

Our findings show that *G. tinctoria* QAs are toxic for non-adapted pea aphid biotypes, whereas the *Genista* biotype is not only surviving QAs in their diet but also sequestering particular QAs. This indicates a large degree of adaption towards its host plant *G. tinctoria*.

Keywords: *Acyrthosiphon pisum; Genista tinctoria; chemical defense; alkaloids; sequestration*

Plant-mediated facilitation of leafminer metamorphosis in the soil

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Abstract

Plants and herbivores are engaged in intimate antagonistic interactions, with plants trying to mount effective defense responses and herbivores attempting to manipulate plants for their own benefit. Here we investigated whether leaf-feeding herbivores can exploit systemically induced plant roots defences to modulate the development of their conspecific soil-dwelling pupae. By using manipulative insect bioassays, we show that tomato (*Solanum lycopersicum*) leaf attack by the American serpentine leafminer *Lyriomiza trifolii* accelerates the development of conspecific pupae in the soil adjacent to the plant via changes in belowground volatiles. This pattern was reversed in the jasmonate-signaling deficient tomato mutant *def-1*. Hormonal, gene expression and chemical analyses showed that *L. trifolii* leaf attack induced defense-associated signaling pathways and the production of root volatiles in a *def-1* dependent manner. Thus, leaf-feeding herbivores can interact with their soil-dwelling pupae via the plant, and jasmonates and root volatiles likely play relevant roles in this phenomenon. This study expands the repertoire of plant-herbivore interactions to herbivory-induced modulation of metamorphosis, with potentially important consequences for plant and herbivore community dynamics.

Key words: jasmonates, pupation, roots, tomato, volatiles.

Race-specific and R-gene-dependent transcriptome responses to pea aphid infestation in *Medicago truncatula*

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Objectives: The *RAP1* quantitative trait locus in *Medicago truncatula* is associated with race-specific resistance to pea aphid (*Acyrthosiphon pisum*). Here we explored transcriptomes and hormone responses of host plants during compatible and incompatible interactions. We also examined suppression of immunity by virulent aphid genotypes, relevant to multiple pest infestations in agricultural environments.

Methods: *Medicago truncatula* near-isogenic lines carrying or lacking *RAP1* were infested with avirulent (PS01) or virulent (N116) aphids. Salicylic acid and jasmonic acid were measured via liquid chromatography-mass spectrometry. Host transcriptomes were compared via RNA-sequencing. Sequential infestation or co-infestation with N116 and PS01 were used to test for resistance suppression.

Results: Salicylic acid accumulated in both resistant and susceptible hosts following PS01 infestation, whereas N116 resulted in lower accumulation. Jasmonic acid showed similar patterns. RNA-Seq revealed differential expression of several classes of transcription factor, most notably *WRKY* and *MYB* genes, and genes for secondary metabolite production including flavonoids, isoflavonoids and saponins. Several genes were upregulated in *RAP1* hosts only in response to avirulent aphids, but another cluster was upregulated in a *RAP1*-independent manner. In contrast, virulent aphids mainly caused down-regulation of genes including members of saponin and flavonoid pathways. Consistent with this negative transcriptional effect, virulent aphids also suppressed *RAP1* resistance, enabling PS01 survival.

Conclusions: Salicylic acid is necessary but not sufficient for *RAP1* defences. Transcriptional reprogramming during compatible or incompatible interactions depends on both host and aphid genotype. However, many genes are upregulated in both types of interactions, suggesting deployment of some ineffective defences. Secondary metabolite induction and suppression may lead to both negative and positive effects on aphids. Notably, defence suppression by virulent aphids enables avirulent aphid survival. Further work is needed to pinpoint the metabolites and other cellular components regulated via the *RAP1*-dependent defence pathway that result in mortality of PS01 but not N116 aphids.

Keywords: Pea aphid, *Medicago truncatula*, salicylic acid, host resistance genes

Plant-insect interactions in the context of biological invasions and biological control

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Biological invasions can be hot spots for rapid evolution. Many things change when a plant invades a new region, including the abiotic environment, the suite of competitors, and the suite of herbivores and other natural enemies. These changes represent shifts in selective pressures that drive evolution. I will evaluate the ecological and evolutionary shifts in the *Verbascum thapsus* (common mullein) system and show that introduced populations are larger and denser than native populations, and that at least part of the difference in performance reflects an evolutionary shift. Introduced populations experience less competition, and lower water availability than native populations, but do not appear to be locally adapted to either of those environmental shifts. Introduced populations also experience less herbivory than native populations, and the community of herbivores has shifted from specialists to generalist. Theory from invasion biology does not deepen our understand how *V. thapsus* responds to these shifts, but classical plant-insect theory, including optimal defense and the evolutionary dilemma model do. Introduced plants invest more in defenses than native plants, but only in the younger, more valuable leaves. By leaving their specialist leaf feeding herbivores behind, they can invest in defenses without risking higher attack. Studying the invasion of *Verbascum thapsus* in North America has helped reveal gaps in our theoretical frameworks surrounding biological invasions that can be filled by bringing plant-insect classics back into focus.

Preference and performance of the biocontrol agent *Aphalara itadori* on invasive knotweed species in the Netherlands

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Background and objective

The knotweed species *Fallopia japonica* and *F. sachalinensis*, introduced from Eastern Asia, and their hybrid *F. x bohemica*, are highly invasive in Europe and North America. *Aphalara itadori*, a psyllid native from Japan specialized on knotweed, has been released in several countries for knotweed biocontrol, with host specificity and control efficiency differing between *A. itadori* populations of different geographic origin. We evaluated performance, oviposition preference and impact on plant growth of the *A. itadori* population from Murakami (Central Japan) that has been released in the Netherlands, on the three knotweed species.

Method

No choice experiments using individually confined plants were carried out to assess *A. itadori* fecundity, population growth and average duration of the immature stage on the three *Fallopia* species. Psyllid impact on plant growth was estimated by comparing growth parameters between infested and control plants eight weeks post-infestation. Oviposition preference was evaluated in a choice experiment where single females were exposed to two plants of different species.

Results

The Murakami population of *A. itadori* performed better on *F. x bohemica* than in the parental species, as indicated by the faster nymph development and the higher final number of adults recorded in this species. Psyllid infestation impacted plant growth in all three species, causing a reduction in rhizome growth in *F. x bohemica* and *F. sachalinensis*, and decreasing stem elongation in *F. japonica*. *Aphalara itadori* females did not display an oviposition preference for any of the host species.

Conclusion

Fallopia x bohemica is the knotweed species in which the Murakami population performs best, suggesting that the hybrid is the most suitable host for the rearing and release of this psyllid population in the Netherlands. However, contrary to the preference-performance hypothesis, females of this population didn't display an oviposition preference for this hybrid species.

Keywords

Knotweed, *Fallopia*, *Aphalara itadori*, weed biological control

Climatic suitability of the invasive aquatic *Iris pseudacorus* L. and its candidate biocontrol agent *Aphthona nonstriata* (Goeze) in the Southern Hemisphere

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Objective

The invasive aquatic *Iris pseudacorus* is the target of a biocontrol programme in the Southern Hemisphere. After preliminary surveys of the plant within its native European range, the flea beetle *Aphthona nonstriata* was prioritized as a candidate biocontrol agent. In this work we model the bioclimatic suitability of both organisms within three invaded ranges: South America, Southern Africa and Australasia. We use the resulting models to discuss the invasion risk of *I. pseudacorus* as well as the potential for control that *A. nonstriata* would provide within each range.

Methods

Species distribution models were computed using the software MaxEnt. Presence-only data were sourced from online datasets, scientific literature and field surveys carried out by the authors. Native and invaded range occurrence records of *I. pseudacorus* distribution were pooled together to better describe the total climatic suitability of the weed. Records were thoroughly cleaned and spatially thinned before model computation. Appropriate bioclimatic variables were selected based on the biology of *I. pseudacorus* and *A. nonstriata*, respectively.

Results

Our results indicate that *I. pseudacorus* holds the potential for range expansion in the Southern Hemisphere. The climatic suitability of *A. nonstriata,* despite covering a smaller area, overlaps well with that of *I. pseudacorus* within these three ranges, highlighting a good probability of establishment and potential control of invasive weed populations.

Conclusion

Iris pseudacorus might still expand its invasive range in the Southern Hemisphere, endangering natural environments and rural systems. The flea beetle *A. nonstriata* shows a good level of climatic suitability within the areas invaded by the weed, and is thus considered a promising candidate for its biological control. This study does not take into account factors other than climate which might limit the spread and establishment of the target organisms, such as abiotic characteristics and biotic interactions present within the each habitat. It provides nonetheless useful information for managing the spread of the current invasion and planning its biological control.

Keywords: *yellow flag iris, iris flea beetle, weed biocontrol, species distribution model, MaxEnt.*

The risk of being eaten: prey metabarcoding reveals which seed traits drive carabid weed seed predation in European wheat fields

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Abstract

Objective

Weed seed removal from arable crops by seed feeding invertebrates affects weed population dynamics and could facilitate natural weed control. Carabid beetles are among the dominant seed feeders native to European farmland and have been associated with weed seed losses. To reveal the actual potential of carabid beetles for weed regulation, we must know which seeds they frequently feed on and understand how this is related to seed traits. We hypothesize that seed traits such as chemical composition and morphological properties, but also availability, can affect the interactions observed in the field.

Method

To study the relative importance of these different seed traits for carabids' seed food choice, we collected the gut content of over 5,000 carabid beetles in 60 commercially managed European wheat fields. The samples were analysed by plant-specific metabarcoding, targeting the nuclear ITS2 region, to reveal the beetles' natural seed diet. This dataset, the biggest of its kind, comprises 120 separate carabid-weed seed food webs, and thus allows to reveal patterns of seed predation including an assessment of how seeds traits correlate with the risk of being consumed by carabid communities. Moreover, predation pressure for seeds with different traits will be calculated by including carabids' energetic need and abundance in the fields.

Results & Conclusion (will be ready for the conference)

The results of the currently ongoing analysis will provide us with information on what seed traits drive carabid-seed interactions to obtain a general understanding which types of weeds are likely to be regulated by carabid beetles in arable fields.

Key Words: weed control, ecosystem services, molecular gut content analysis, food web ecology

Biological control in circular agriculture - Using insect side-streams to increase crop growth and parasitoid recruitment

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Objective

The production of insects as food and feed is increasing rapidly. Therefore, also the resulting side-streams, exuviae and frass, are increasing. To promote circularity in agriculture, these side-streams can be used as soil amendment for crops. Besides adding extra nutrients, the side-streams may also induce defences in the crops and thereby help in protecting crops against pest insects. In this study we tested whether soil amendment with insect exuviae can increase parasitoid recruitment in the field.

Method

Brussels sprouts plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus) were sown on field soil treated with exuviae from mealworm (*Tenebrio molitor*), house cricket (*Acheta domesticus*) or black soldier fly larvae (*Hermetia illucens*). Parasitoid attraction was measured in a Y-tube olfactometer set-up, using the aphid *Brevicoryne brassicae*, the caterpillar *Plutella xylostella* and their respective parasitoids *Diaeretiella rapae* and *Diadegma semiclausum*. Subsequently, a field experiment was conducted, testing for plant growth and recruitment from the naturally occurring parasitoid population to introduced *B. brassicae* and *P. xylostella*.

Results

Plants growing on soil treated with mealworm or cricket exuviae attracted more parasitoids in the Y-tube olfactometer. In the field experiment, a significantly higher proportion of parasitized herbivores were recovered from the plants growing on exuviae-treated soils than on plants growing on untreated soil. Furthermore, the plants had a higher dry shoot biomass, more leaves and a broader stem base, when growing on soil treated with insect exuviae.

Conclusion

With this study we show that insect exuviae can be used as a soil amendment in agriculture, not only to promote crop growth as a fertilizer, but also to enhance biological control by increasing the recruitment of parasitoids.

Key words

Parasitoid recruitment, biological control, insect side-streams, circular economy, field study

Chemical communication in inter-specific ecological interactions

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Information transfer via chemical cues and signals plays a key role in ecological interactions among species. My program is exploring these communicative interactions in a range of systems, with a particular focus on plants and insects.

In this talk, I will discuss recent findings in three areas: (i) plant responses to olfactory cues associated with the threat of herbivory (ii) effects of pathogen-induced changes in host odors on interactions between plants and insect vectors; and (iii) bee-derived cues that influence plant flowering time.

A mosaic of induced and non-induced branches promotes variation in plant traits, predation and leaf palatability to insect herbivores

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Forest canopies are a complex three-dimensional environment showing high variation in both biotic and abiotic conditions that affect trees and their traits. The resulting variation in tree physical and chemical traits creates niches for a multitude of insect herbivores and predators harboured by canopy trees. Here, we hypothesized that branch-localized defence induction and vertical stratification in mature oaks are two major sources of variation in their leaf chemistry and physical traits and that their effects can cascade across trophic levels.

We tested for the effects of branch-localized defence induction and vertical stratification on tree defensive traits, herbivores, and predators using a series of field and laboratory experiments. Specifically, we combined manipulation of plant defences, predation monitoring, laboratory trials with insect herbivores, and sampling of natural herbivore assemblages from a canopy crane.

Both induction and vertical stratification affected tree defensive traits, but the effect of defence induction was generally stronger and affected both direct and indirect defences. These effects cascaded to insect herbivores directly by affecting their preference in food choice trials or indirectly by increasing predation in the canopy that negatively correlated to herbivore abundance. There was also high variation in composition of herbivore assemblages among oak individuals and strong effects of seasonality.

In turn, we show that forest canopies are a dynamic system where multiple factors contribute to their overall diversity and variation in traits and biotic interactions. Intraspecific variability between tree individuals and predation may be the ones with particularly strong effects on diverse insect assemblages in canopies of temperate forests.

Key words: canopy; diversity; induced defences; trophic interactions, vertical stratification

The effect of parasitized aphids on plant-mediated interaction networks

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Objective:
Parasitism is known to alter the physiology and behaviour of herbivores. This affects herbivore induced plant-responses and subsequently plant-mediated interaction networks through changes in herbivore induced plant volatiles (HIPVs). Hyperparasitoids are known to eavesdrop on these changes to locate their parasitoid host concealed inside herbivores. However, this has never been shown in an aphid-parasitoid-hyperparasitoid system. Here, we studied whether parasitism affects the feeding behaviour of aphids, changes induced plant responses and if an aphid hyperparasitoid can successfully eavesdrop on these changes and detect its host through HIPVs.

Method:
We used a wild cabbage-based food web, involving the most common players and trophic interactions as observed in the field. Hence, we selected the cabbage aphid *Brevicoryne brassicae,* its common parasitoid *Diaeretiella rapae* and the true aphid hyperparasitoid *Alloxysta fuscicornis*. We infested plants with either healthy or parasitized aphids and studied: a) Aphid feeding behaviour with the electrical penetration graph method (EPG). b) Induced plant responses at transcript level with qPCR. c) The preference of the hyperparasitoid to the volatiles of plants with either healthy or parasitized aphids using the Y-tube olfactometer.

Results:
Parasitized aphids were found to have a higher frequency and longer duration of xylem feeding behaviour compared to healthy aphids. Both healthy and parasitized aphids induce the Salicylic Acid signalling gene *PR1*, but only healthy aphids manage to suppress the Jasmonic Acid biosynthesis gene *LOX2*. Finally, the hyperparasitoid preferred volatiles of plants induced with (parasitized) aphids over clean control plants and preferred those with parasitized aphids over healthy aphids.

Conclusion:
We show that the aphid hyperparasitoid *A. fuscicornis* is able to use plant volatiles to locate its parasitized aphid host. Underlying this are changes in aphid feeding behaviour, plant transcript and variation in volatiles.

Keywords:
Multitrophic-interactions, host-location, feeding behaviour, plant volatiles

Influence of mycorrhizal symbiosis and fertilization on the performance of *Spodoptera exigua*: are effects mediated by a change of the insect gut microbiota?

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Objective: The aim of this study was to investigate the effect of arbuscular mycorrhizal fungi (AMF) on *Spodoptera exigua* larval performance and gut microbiota under two different nitrogen (N) fertilization levels. We hypothesize that AMF colonization (1) affects the performance of *S. exigua* by alteration of the larval endosymbionts, and (2) influences the N availability and its consequences on this multiple partner's interaction.

Methods: Maize (*Zea mays*) plants were used as host plants to conduct a full factorial design to study the effects of the interaction between the two factors (AMF inoculation and N fertilization level) on the generalist chewer *S. exigua*. Two different species of AMF were tested. Larval survival was daily recorded during 5 days. Surviving larvae were collected to analyze their gut bacterial diversity using 16S DNA metabarcoding sequence in Illumina Miseq. Vegetal material was harvested for further nutritional content analyses (Dumas method and GC-MS).

Results: (1) The effects of the interaction between the AMF inoculation and the N fertilization level on the survival of the larvae are AMF species-dependent. (2) Bacterial communities of *S. exigua* are not highly diverse but significant differences in abundance and occurrence are detected between treatments. Results also suggest differences in terms of species richness between the variables. (3) The carbon:nitrogen ratio of the plants is affected by the interaction between the studied factors.

Conclusion: Our results suggest that the AMF inoculation-N fertilization regime interaction mediate changes of the gut microbiota of *S. exigua* with potential impact on the larval performance. These results might contribute to a better understanding of the biotic and abiotic context-dependency of mycorrhiza-induced resistance allowing for the development of more robust biocontrol of insect pests.

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Direct and insect-mediated effects of pathogens on plant growth and fitness

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Objective. Plants are attacked by a large diversity of pathogens. These pathogens can affect plant growth and fitness directly, but also indirectly by inducing changes in the host plant that affect interactions with beneficial and antagonistic insects. Yet, we lack insights into the relative importance of direct and indirect effects of pathogens on their host plants, and how these effects differ among pathogen species.

Method. In this study, we examined four fungal pathogens on the wood anemone (*Anemone nemorosa*). We used field observations to record the impacts of each pathogen species on plant growth and fitness throughout the season, and experimental hand-pollination and insect feeding trials to assess whether fitness impacts were mediated by pathogen-induced changes in plant-pollinator and plant-herbivore interactions.

Results. Three out of four pathogens negatively affected plant size, and pathogens differed strongly in their effect on plant architecture. Infected plants had lower fitness, but this effect was not mediated by pollinators or herbivores. Even so, two out of four pathogens reduced herbivory on anemones in the field, and we found negative effects of pathogen infection on herbivore preference and performance in feeding trials.

Conclusion. Our results are of broader significance in two main respects. First, we demonstrated that pathogens negatively affected plant growth and fitness, and that the magnitude of these effects varied among pathogen species, suggesting that pathogens constitute important selective agents that differ in strength. Second, direct effects on plant fitness were more important than effects mediated by beneficial and antagonistic insects. In addition, even though we did not detect insect-mediated effects on plant fitness, the negative effects of some pathogens on herbivore preference and performance indicate that pathogen communities influence the distribution and abundance of herbivores.

Keywords: Insect herbivores — Plant–pathogen–insect interactions — Plant performance — Pollinators — Rhizomatous clonal plants

The Community Ecology of Herbivore Regulation in an Agroecosystem: Lessons from Complex Systems

John Vandermeer

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Whether an ecological community is controlled from above or below remains a popular framework that continues generating interesting research questions and takes on special importance in agroecosystems. In this talk I describe the regulation from above of three coffee herbivores, a leaf herbivore (the green coffee scale, Coccus viridis), a seed predator (the coffee berry borer, Hypothenemus hampei), and a plant pathogen (the coffee rust disease, caused by Hemelia vastatrix) by various natural enemies, emphasizing the remarkable complexity involved. I emphasize the intersection of this classical question of ecology with the burgeoning field of complex systems, including references to chaos, critical transitions, hysteresis, basin/ boundary collision, and spatial self-organization, all aimed at the applied question of pest control in the coffee agroecosystem.

Systemic plant responses to belowground herbivory deter aboveground herbivores

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Upon insect herbivore attack, plants initiate a cascade of defensive responses. Such responses alter plant metabolic activity locally, and can also systemically affect distal, non-attacked plant tissues. Belowground herbivory damages plant roots, but the systemic effects on aboveground plant tissues and cascading consequences for aboveground herbivores remain poorly understood. Therefore, our objective was to determine how belowground striped cucumber beetle (*Acalymma vittatum*) larval herbivory alters aboveground squash (*Cucurbita pepo*) plant tissues and interactions with aboveground squash bugs (*Anasa tristis*) and adult beetles. We predicted belowground beetle larvae systemically manipulate plant tissues to deter aboveground herbivores, as larvae compete for shared plant resources with both squash bugs and adult beetles. To investigate our objective, we conducted a series of greenhouse and field experiments comparing undamaged, control plants and larvae-damaged plants. We allowed larvae to feed for 48 hours and we collected aboveground plant volatiles in the greenhouse from control and larvae-damaged plants, as volatiles can have deterrent effects on foraging herbivores. Larvae-damaged plants emitted higher amounts of (*E*)-β-ocimene aboveground than controls. In both the field and greenhouse, we examined adult beetle feeding and foraging behaviour and found adults avoided feeding and settling on larvae-damaged plants. We also determined how squash bugs responded to belowground herbivory in the greenhouse and discovered squash bugs avoided settling and laying eggs on larvae-damaged plants. To complement these findings, we developed synthetic (*E*)-β-ocimene dispensers to isolate (*E*)-β-ocimene effects on foraging adult beetles and squash bugs, and we found this compound deterred squash bugs but not adult beetles. Taken together, our results indicate belowground herbivory modifies systemic plant tissues, ultimately deterring aboveground herbivores. Although volatiles play a part in aboveground herbivore avoidance of larvae-damaged plants, volatile emissions alone do not fully explain these interactions and we anticipate systemic changes in nutrients also shape aboveground herbivore behaviour.

Keywords: induced systemic defences; plant volatiles; belowground herbivory; above/belowground interactions

Title: Parasitoids response to extreme temperature events are driven by the structure of their surrounding community.

Affiliations:

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Objective

Understanding the impacts of extreme high-temperature events (EXT) on species interactions is of utmost importance to better predict the effects of climate change on ecosystem functioning. Little is known about the effects that the interacting community has on the response of a targeted organism to EXT. Here we present empirical findings on the parasitism performance of two sympatric parasitoid species foraging at different temperatures and species density levels and discuss these findings in the context of population and community dynamics.

Method

We performed a set of two combined foraging experiments using a worldwide herbivorous pest, *Plutella xylostella* (Lepidoptera: Plutellidae) and two of its larval endo-parasitoid *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* (Hymenoptera: Braconidae). We placed 30 *P. xylostella* larvae on fully grown *Brassica oleracea* (Brassicaceae) plants at 22°C, 27°C or 33°C and released 1, 2,4 or 8 parasitoids for three-hour foraging before recapture. The host-plant system was kept until insect pupation, and species offspring were sorted and counted from their pupae. The data were analysed using a generalised linear model to measure the effect of density and temperature on the parasitism rate of each species foraging alone or in combination.

Results

When foraging alone, both species parasitism increased at a decelerating rate with increasing parasitoid density suggesting mutual interferences between individuals at high density. When foraging with *C. vestalis*, *D. semiclausum* parasitism performance was not affected at 22°C but lowered at 27°C and 33°C. In comparison, *C. vestalis* displayed reduced performance in the presence of *D. semiclausum* at all temperatures. The parasitism achieved by each species tended to converge as the temperature increased.

Conclusion

In the context of changing climate, the species response to the increase in magnitude and frequency of EXT is driven by the community structure.

Keywords: Extreme high temperature; Species interactions, Parasitism, *Plutella xylostella*

Volatile interaction between different barley cultivars affects aphid feeding behaviour

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Abstract

Recent studies demonstrated that cultivar mixture can reduce aphid population development. However, it is still unknown which underlying mechanisms may contribute to this phenomenon. The main objective of this study is to investigate the effects of volatile interactions between different barley cultivars on the aphid feeding behaviour. Two cultivars of spring barley plants (*Hordeum vulgare L.*) were used Salome and Fairytale in the laboratory bioassays. One seed of each cultivar were sown per pot and at two-leaf stage plants were transferred in the twin cages for 5-days where Salome were exposed to Fairytale and clean air as a control. We used electrical penetration graph (EPG) to test the effect of exposure to neighbour volatiles on feeding behaviour of bird cherry-oat aphids (*Rhopalosiphum padi* L.). Volatiles from neighbouring cultivar may induce response in the receiving cultivar affecting aphid feeding behaviour. Such aphids spend significantly more time on plant surface without trying to probe the plant tissue on exposed plant than unexposed. Significant decrease in duration of phloem phase (E12) and sustained feeding phase (sE2) were recorded when Salome were exposed to Fairytale showing that volatile induced effects case difficulty for aphids to feed. This study demonstrated that exposure of one barley cultivar to volatiles from the other one may change host plant quality, which can be one of possible explanation for the observed reduced aphid population development in the field.

Keywords:
barley, aphid, cultivar mixture, insect-plant interaction, EPG

Tree species composition and water availability influence direct and indirect defences in *Quercus ilex*

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Abstract

Plant species richness is known to affect plant-herbivore interactions. Damage caused by insect herbivores has often been reported to be lower in species mixtures than in monocultures both in agriculture and forestry systems. This phenomenon, known as associational resistance, may rely on the effects of species composition on plant traits that act as direct defences against insect herbivores (e.g., phenolic compounds) or as indirect defences involved in the attraction of herbivore natural enemies (e.g., volatile organic compounds-VOCs). In addition, abiotic conditions (e. g., resource and water availability) may also modify defensive investment and thus affect the outcomes of associational resistance. We used a long-term experimental trial to explore whether tree composition and water availability affected herbivory and direct and indirect defences in *Quercus ilex*. To that aim, we measured leaf herbivory by chewing insects and analysed phenolic compounds and VOCs in *Q. ilex* trees growing in stands differing in tree composition (*Q. ilex* + *B. Pendula*, *Q. ilex* + *P. pinaster* and *Q. ilex* + *B. pendula* + *P. pinaster*) and under different water availability conditions (irrigated vs control). We did not observe a significant effect of tree composition or irrigation on leaf herbivory in *Q. ilex*. However, tree composition significantly affected the production of both direct and indirect defences. In particular, *Q. ilex* trees growing in stands with *P. pinaster* had lower concentration of both direct and indirect defences. Irrigation also affected the production of indirect defences in *Q. ilex*, but only when growing with *P. pinaster*, where the concentration of VOCs was lower on irrigated trees. Our results suggest that reduced defensive investment in irrigated stands with higher species richness may operate in opposite direction than associational resistance and thus counter the beneficial effects of species richness in *Q. ilex*.

Keywords: Chemical defences, insect herbivory, *Quercus ilex*, tree diversity, water availability

The Parasite Paradox: host repertoires and the diversification of butterflies

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Parasites – including plant-feeding insects – are known to be highly specialized and conservative in their host use. Yet there is much evidence for rapid changes in host use, the current pandemic being but one extraordinarily striking example. This is the Parasite Paradox: parasitic organisms appear to shift hosts much more easily than they should. I argue that the reason that the paradox has arisen in the first place is that we have collectively had a somewhat simplistic view of how these interactions evolve. I further suggest that the concept of host repertoires can facilitate a better understanding of the processes that give rise to these seemingly incompatible patterns, and how they in fact follow as a natural consequence of the particular way that host repertoires evolve and are maintained. Finally, I will discuss how the concept of host repertoires can assist in understanding the diversification of butterflies, and potentially other parasitic interactions.

PHYLOGENETIC CONSTRAINTS ON THE EVOLUTION OF FLORAL SCENTS IN A NURSERY POLLINATION MUTUALISM

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Most studies of chemical mediation between plants and pollinators stress the direct impact of selection by pollinators on the composition of floral scents. Nevertheless, phylogeny may constrain scent composition and thereby the evolution of the emitted signal. Using a model system for obligate pollination interactions, those between figs and their species-specific pollinating fig wasps, we studied whether phylogenetic history constrains the composition of plant chemical signals mediating interactions with pollinators. In this 'nursery pollination mutualism', the pollinators can breed only in receptive figs of their host tree, which depends in turn on the wasp as its sole pollinator. The encounter of the pollinator and the receptive fig is mediated by volatile organic compounds (VOCs). We collected floral scents from receptive figs using in situ headspace extraction of odours from 32 species of several sub-genera of Ficus from different tropical and subtropical regions, and analysed their chemical composition by gas chromatography / mass spectrometry (GC-MS). Using phylogenies available for *Ficus*, we analysed the phylogenetic signal in semi-quantitative patterns of floral- scent data using phylogenetic principal component analysis and several multivariate indices of phylogenetic signal. Our results revealed a strong phylogenetic signal in the VOCs emitted by receptive figs, probably due to constraints in the biosynthetic pathways of volatile compounds. Using the same analysis, we found no effect of the pollinator phylogeny. These findings constitute one of the first demonstrations, on a wide scale, that phylogenetic constraints play a significant role in the diversification of VOCs signals emitted by receptive flowers.

KEYWORDS
Floral scents, mutualism, pollination, phylogeny, chemical mediation

Evolutionary changes in an invasive plant support the defensive role of plant volatiles

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ABSTRACT

The key role of foliar volatiles in the interactions among plants, herbivores, and their natural enemies is widely recognized, but whether the volatiles have an evolved tritrophic signaling function remains hotly debated. Unharmed leaves constitutively release small amounts of volatiles, but their levels increase considerably when the leaves are damaged by herbivorous arthropods. We investigated the respective defensive roles of constitutive and inducible volatiles by studying their evolutionary changes in the common ragwort, Jacobaea vulgaris. This European plant invaded several continents, where it evolved for many generations in the absence of specialized herbivores and their natural enemies. We found that, compared to native ragworts, invasive plants release higher levels of constitutive volatiles, but considerably lower levels of herbivore-induced volatiles. As a consequence, invasive ragwort is more attractive to a specialist moth, but avoided by an unadapted generalist moth. Importantly, conform to the indirect defense hypothesis, a specialist parasitoid was much more attracted to caterpillar-damaged native ragwort, which mirrored higher parasitism rates in a field trial. Evidently, we concluded that the evolution of foliar volatile emissions is indeed driven by their direct and indirect role in defenses against insects.

KEYWORDS: Jacobaea vulgaris, Tyria jacobaeae, Cotesia popularis, constitutive plant volatiles, herbivore-induced plant volatiles

Mutualism promotes range expansion in both plant and insect partners

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Type of presentation requested: Oral presentation

The ability of a species to colonise new habitats can be viewed as a measure of its ecological success. Interacting with a mutualist may facilitate range expansion by increasing the chance of survival in a novel environment, compared to a non-mutualist. We investigated the effect of mutualism on range size in both partners in the ant-extrafloral nectary (EFN) mutualism in legumes (Fabaceae) in which ants act as bodyguards against herbivores in return for nectar from the EFNs on host plants. The majority (~22%) of known plant species possessing EFNs are legumes.

Since ants are ubiquitous, we predicted that bearing EFNs facilitates range expansion in plants due to protection from herbivory by visiting ants in new ranges. Correspondingly, we predicted that ant species visiting EFNs are more successful at colonizing new habitats since they can utilize a distinct food resource.

Using published datasets on legume range size, EFN presence and a legume phylogeny, we found that legume species with EFNs have higher invaded range sizes than species that lack EFNs. Similarly, using a text-mined list of ant species that visit EFNs, data on ant native and invaded range size and an ant phylogeny (all obtained from published literature), we found that ant species that are likely to visit EFNs have both higher native and invaded range sizes.

This suggests that participating in mutualism leads to a “cooperate-and-expand” effect when studied on a macroevolutionary scale. We acknowledge that well-studied ant species may be overrepresented in the text-mined dataset, but similar trends in both native and invaded range size of ant species that visit EFNs suggest that EFNs do indeed facilitate range expansion in ants. This is one of few studies that examine the effects of mutualism on both partners in the macroevolutionary scale.

Key words:

Mutualism, ant, extrafloral nectary, range expansion, benefits, legumes, macroevolution

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Extreme gene and intron loss in the miniature genome of the tomato russet mite

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Objective

The tomato russet mite, *Aculops lycopersici*, is a tiny metazoan herbivore, merely 200 micrometers long, and an important pest on tomato. It belongs to the Eriophyoideae (Arthropoda: Chelicerata: Acari) and is able to suppress tomato defenses, resulting in a weakened host that it rapidly overcomes. The genome of *A. lycopersici* was sequenced to investigate whether there are genomic features associated with the mite's size and lifestyle.

Method

The *A. lycopersici* genome was sequenced using Roche/454 technology, while Newbler software was used for assembly. Genome completeness was assessed by both *k*-mer and BUSCO analyses and gene prediction was performed using a combination of EuGene and Cufflinks. The latest versions of the Malin and CAFE software were used for intron and gene family analysis, respectively.

Results

Our sequencing and annotation of the *A. lycopersici* genome revealed that, mirroring its minute physical stature, *A. lycopersici* has the smallest genome - 32.5 Mb - of a free-living animal reported to date. As compared to other arthropods, genome reduction was accomplished by reduced gene number, short intergenic distances, and few repetitive sequences. In addition, massive intron loss was apparent – more than 80 % of genes lack introns – a feature more reminiscent of some yeast genomes than of other animal genomes. Gene families are almost uniformly reduced, and many arthropod-conserved genes are absent, including the Hox segmentation gene *proboscipedia*. Furthermore, genes in a number of families associated with plant host use by arthropods, including those involved in detoxification and chemosensory perception, were minimal in number.

Conclusion

Our work sheds light on genomic features associated with arthropod host specialization, as well as extreme physical miniaturization in metazoans. Furthermore, the genome provides an important resource for investigating the molecular basis of *A. lycopersici*'s ability to potently suppress the defenses of a major crop plant.

Keywords: eriophyids**,** microarthropods, genome streamlining

The sweet taste of nectar: novel mechanisms for taste coding revealed in bees

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Floral nectar is a carbohydrate-rich reward created by plants to entice pollinating insects to visit. Bees, like all other insects, use their sense of taste to make rapid decisions about food quality. Carbohydrates are critical nutrients detected by sugar-sensing gustatory neurons (GRNs) in insects that elicit action potentials (spikes) in response to stimulation. I will discuss the specialized means that bees have for detecting sugars on their mouthparts. These include my lab's unique discovery that bursts of coherent spikes are produced when bee GRNs are stimulated by sugars of high metabolic value. We have recently discovered that the majority of neurons (three out of four) in the sensilla on the galea of the bee's mouthparts respond to sugars. Our data show that temporal information about spike timing from the whole GRN population across the mouthparts contributes to information coding about the identity of sugars. I will discuss how these mechanisms contribute to the control of feeding and to the discrimination of floral nectar.

Desert time: Phylogeny and abiotic conditions shape the diel floral emission patterns of desert Brassicaceae species

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A significant facet of floral scent is diel fluctuations in emission patterns, particularly in regions where pollinators are scarce. Here, we reveal the interplay between environment and phylogeny in shaping temporal variations in floral scent and their relation to pollinators. In this research, we measured the light/dark volatile emission of 17 desert Brassicaceae species using a headspace gas chromatography-mass spectrophotometer. Then, we coupled this data with environmental and phylogenetic data to generate comprehensive models, exploring the individual and combined impacts of these predictors on diel emission patterns. We also investigated these patterns in greater detail by conducting high-resolution emission measurements for a subset of genetically distant species with contrasting phenotypes and recorded pollinator visitations. We found that while diel shifts in emission magnitude and richness are strongly affected by genetic relatedness, they also reflect the environmental conditions under which the species grow. Light/dark emission ratios were negatively affected by winter temperatures, impacting plant physiology and insect locomotion; and by sandy soils, known to exert water stress that tempers with diel metabolic rhythms. The biosynthetic origins of the compounds explain their corresponding emission patterns, which are correlated to pollinator behavior. Using multidisciplinary chemical and ecological approaches, we uncover and differentiate the main factors beyond mutualist interactions to shape floral scent diel fluctuations, highlighting their consequences under changing global climate.

Keywords: floral scent; volatile; pollinator; environment; arid

ABSTRACT TITLE

Specificity of pollinators between syntopic species of the genus *Ceropegia* (Apocynaceae) sharing the same pollination syndrome

AFFILIATIONS
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ABSTRACT TEXT

Objective

Flowers in the genus *Ceropegia* L. (Apocynaceae) do not reward pollinators: pollination is based on deceit. Inside the flower, pollen grains are grouped within five pollinia, limiting transfer opportunities. In case of syntopy with species sharing the same pollination syndrome, the risk of losing its pollinia inside another species should induce a selection pressure on pollinator specificity.

Method

We collected data on basic pollination aspects of four *Ceropegia* species occurring syntopically in Thailand, analysed floral scent by gas chromatography linked to mass spectrometry (GC/MS), and determined compounds responsible for pollinator attraction in bioassays.

Results

All pollinators found in the flowers were Diptera from Milichiidae and Chloropidae families, known as kleptoparasites. The floral scents and the scent of a true bug found on site – supposed to be the model mimicked by the flowers – belonged to the same family. This suggests a kleptomyiophilous syndrome: the flower attracts Diptera feeding on the hemolymph of insects captured by arthropod predators. The four plant species showed clearly distinct pollinator communities and floral scents, yet the pollinator attraction with synthetic odors was less specific.

Conclusion

The pollinator specificity in a syntopic context was confirmed, and implied, at least in part, the floral scents.

KEYWORDS Diptera ; Kleptomyiophily ; Floral scent ; Specialization ; Thailand

ABSTRACT TITLE:

Developing a taste for sweets: Sugar detection and preference in *Manduca sexta* caterpillars and adults reflects leaf and nectar feeding lifestyles

AFFILIATIONS

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ABSTRACT TEXT

Metamorphosis enables individual plant-feeding insects to occupy vastly different ecological niches and often drastically changes the relationship with their host-plant. *Manduca sexta* caterpillars are voracious herbivores on different solanaceous plants, while the adult hawkmoth is one of the main pollinators of these plants. Here, we show how this change from an antagonistic herbivore to a mutualistic pollinator is reflected by the sugar taste of caterpillars and hawkmoths.

First, the feeding preferences of caterpillars and hawkmoths towards different concentrations of sucrose, fructose and glucose were tested. Additionally, electrophysiological responses of different taste neurons to these sugars were quantified. The expression of sugar receptor genes in the caterpillar mouthparts and the moths' proboscis was also analysed. Currently, we are analysing the sugar profile in the leaves and flower nectar of plants that are attacked by *M. sexta* caterpillars, as well as being pollinated by the moth.

While caterpillars consumed significant amounts of a glucose supplemented agar-diet, hawkmoths did not take-in any glucose solution. Conversely, caterpillars did not feed from an artificial diet containing fructose, while it initiated a strong feeding response in moths. Sucrose elicited feeding behaviour in both life stages. Caterpillars and moths also differed in their preference for different sugar concentrations, with caterpillars preferring lower sugar concentrations and moths' higher concentrations. For moths, feeding preferences closely resembled the responses of taste neurons on the proboscis tip, whereas for caterpillars the taste neurons also responded to fructose, which did not trigger feeding. This broader response spectrum in the caterpillar neurons matched with their more diverse sets of receptor genes. Our final aim is to correlate these findings with the sugar composition in leaves and flower nectar.

Ultimately, more knowledge on the development of sensory systems in insects will help to better understand the complexity of both mutualistic and antagonistic insect-plant interactions.

KEYWORDS

Sugar taste, Pollination, Herbivory, *Manduca sexta*

Pollination by wild bees in sunflower crops: a metabarcoding approach

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Objective: In this study, we aim to test whether a DNA metabarcoding approach is an efficient method to describe the pollination activity of wild bees, including their use of wild flowers, is to say, the pollinated plants in sunflower crops in Chizé (France). Moreover, we aim to test whether ecological factors, such as the functional traits from the insect or agricultural characteristics, influence the choice of plants pollinated by wild bees.

Methods: Wild bees were collected in sunflower crops in Western France. Pollen DNA was extracted from the specimens using a washing protocol and an ITS metabarcoding library was prepared and sequenced with Illumina MiSeq. A bioinformatic toolbox was used to obtain the DNA sequences of the pollinated plants and estimate absolute abundance of each plant visited by each individual bee. Finally, biodiversity metrics, Generalized Linear Models (GLMs) and species richness were computed to test which factors influence the choice of wild bees.

Results: Our results show that the method is efficient to infer plant visitations by wild bees. The most commonly found plants were in the families Asteraceae and Brassicaceae. Biodiversity metrics, GLMs and species richness results show differences between the plants chosen by the bees in relation to the species of bee and its sex, the weed cover, the average height of the sunflower plants, the sampling period (flowering or post-flowering) and the type or crop (organic or conventional).

Conclusion: This study demonstrates the efficiency of our genomic approaches to study pollination in wild bees. Our results provide valuable information about floral resources used by wild bees under different conditions and inform the conservation of these key species, which provide essential pollination services in crop ecosystems.

Key words: Wild bees, sunflower, DNA metabarcoding, pollen, biodiversity.

How multifunctional secondary metabolites shape plant-herbivore interactions

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Plants produce diverse sets of secondary (or specialized) metabolites, many of which are classified as defense compounds. Work over the last decades has shown that secondary metabolites have functions beyond defense, including roles in signaling, growth and development. However, our understanding of how this multifunctionality affects interactions between plants and herbivores is limited. In my presentation, I will explore how multifunctionality influences plant herbivore interactions by discussing our work on benzoxazinoids, which are produced abundantly by grasses such as wheat and maize. We find that benzoxazinoids act as direct defenses, within-plant defense signaling molecules, microbiome modulators, and siderophores. This multifunctionality leads to 1) tradeoffs between caterpillar and aphid resistance, 2) interactions between soil chemistry and leaf herbivore resistance and 3) multifunctional adaptations of specialist herbivores. Thus, accounting for the multifunctionality of plant secondary metabolites can improve our understanding of their context-dependent impact on plant herbivore interactions, with implications for their ecology, evolution and pest control potential.

A horizontal gene transfer event has empowered whiteflies with the ability to neutralize common plant defense compounds

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Objective

Our research aims to unravel the mechanisms and evolutionary processes that allow herbivorous pests to develop resistance traits against plant defenses. This knowledge may then be used for the development of pest control strategies. We study the whitefly *Bemisia tabaci*, a cosmopolitan, highly polyphagous agricultural pest that vectors several serious plant pathogenic viruses, thereby causing tremendous economic losses. It is not well understood why this insect can survive on more than 600 different host plants.

Methods

Using a combination of bioinformatic tools, molecular and chemical analytical techniques as well as performance assays, we studied the molecular mechanisms that allow *B. tabaci* to overcome plant defenses.

Results

The experiments revealed that the whitefly has acquired a plant gene through an extraordinary horizontal gene transfer event. Homologs of this phenolic glucoside malonyltransferase gene, named *BtPMaT1*, were found in most plants but not in any other insect. *BtPMaT1* allows whiteflies to neutralize various phenolic glucosides, common plant defense compounds, may may explain their extreme polyphagous nature of whiteflies. The function of *BtPMaT1* was confirmed with gene silencing experiments. For one such experiment, tomato plants were genetical modified to produce double stranded RNA that, when ingested by the whiteflies, silences the *BtPMaT1* gene. Almost all whiteflies died on these transgenic tomato plants, whereas other arthropods that fed on the plants were unaffected.

Conclusions
We show that more than 35 million years ago a gene from plants was horizontally transferred to *B. tabaci*. It allows this whitefly to neutralize phenolic glucosides and probably other plant defense compounds. The work reveals a novel evolutionary scenario, whereby herbivores can acquire foreign genetic material to evolve traits that allow them to cope with plant defenses. Transforming crops with specific gene silencing properties that target *BtPMaT1* may offer an effective way to control an exceedingly important worldwide pest.

Literature: Xia J., Z. Guo, Z. Yang, H. Han, S. Wang, H. Xu, X. Yang, F. Yang, Q. Wu, W. Xie, X. Zhou, W. Dermauw, T.C. J. Turlings, and Y. Zhang (2021). Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. Cell Online: https://doi.org/10.1016/j.cell.2021.02.014

The effects of red to far-red light ratio on plant-insect interactions across trophic levels

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LED lights provide farmers with the possibility to alter the colour of light and can use that to influence crop growth and quality. But it also influences plant-insect interactions and might therefore have a role in pest management.

The ratio of red to far-red light (R:FR) is an important signal for plants for the detection of neighbours. Low R:FR means that R light is being “stolen” by competitors, and induces the elongation of stems. This rapid growth response goes at the expense of plant defences, and leaves the plant vulnerable to herbivory. At the same time, plants become more attractive to natural enemies, indicating a switch from direct to indirect defences. In our research we aim to discover if the addition of R light, a common practice in greenhouses, can strengthen the resistance of tomato (*Solanum lycopersicum*) against herbivores, and how this influences plant-predator interactions.

We exposed tomato plants to three different R:FR ratios (0.5, 1.2 and 5.0) and measured the performance of four herbivore species belonging to different feeding guilds (*Manduca sexta, Tetranychus urticae, Myzus persicae* and *Trialeurodes vaporariorum*). We also measured the preference of the predatory mite *Phytoseiulus persimilis* for *T. urticae*-infested plants exposed to the three R:FR treatments, using pairwise choice assays in a Y-tube.

We observed that exposure to low R:FR (0.5) increased the performance of all four herbivore species compared to a R:FR of natural sunlight (1.2). However, higher R:FR (5.0) reduced the performance of only *M. sexta*. We also observed that the predator *P. persimilis* preferred prey-infested plants when exposed to low R:FR (0.5) compared to the other treatments, with no differences between daylight (1.2) and high R:FR (5.0).

Our results show the importance of FR light in plant-insect interactions, and show no distinct effects of supplemental R light on plant-herbivore and plant-predator interactions.

Keywords: LEDs, herbivore performance, R:FR, natural enemies.

Exposure to (*Z*)-11-hexadecenal increases *Brassica nigra* susceptibility to subsequent herbivory.

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Objective

It is well established that plants emit, detect, and respond to volatile organic compounds (VOCs); however, knowledge on the ability of plants to detect and respond to volatiles emitted by non-plant organisms is limited. The study I will present explores whether exposure to a main component of *Plutella xylostella* female sex pheromone namely (*Z*)-11-hexadecenal [(*Z*)-11-16:Ald] induced detectable early and late stage defence-related plant responses in *Brassica nigra.*

Method

We exposed *B. nigra* plants to biologically relevant levels of vapourised (*Z*)-11-16:Ald released from a loaded septum for 24h and collected volatile emission rates from both control and (*Z*)-11-16:Ald-exposed plants before and after *P. xylostella* larvae were allowed to feeding on the plants for 24h. Next, we examined the effects of the 100ppm of (*Z*)-11-16:Ald in liquid state on several early stages of plant defence-related responses .

Results

Exposure to (*Z*)-11-16:Ald induced a change in volatile emissions of receiver plants after herbivore attack and increased the leaf area consumed by *P. xylostella* larvae. Further in vitro experiments showed that exposure to 100ppm of (*Z*)-11-16:Ald in liquid state induced depolarisation of the transmembrane potential (Vm), an increase in cytosolic calcium concentration $[Ca^{2+}]_{cyt}$, production of H_2O_2 and an increase in expression of reactive oxygen species (ROS)-mediated genes and ROS-scavenging enzyme activity.

Conclusion

The results suggest that exposure to volatile (*Z*)-11-16:Ald increases the susceptibility of *B. nigra* to subsequent herbivory. This unexpected finding suggests alternative ecological effects of detecting insect pheromone to those reported earlier. Experiments conducted in vitro showed that high doses of (*Z*)-11-16:Ald induced defence-related responses, but further experiments should assess how specific the response is to this particular aldehyde.

Keywords: (*Z*)-11-hexadecenal, *Plutella xylostella* (Diamondback moth), volatile organic

ABSTRACT TITLE

Short-term exposure to silicon rapidly enhances plant resistance to insect herbivory

AFFILIATIONS

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ABSTRACT TEXT

Objective

Silicon (Si) deposition in plant tissues is known to negatively impact many insect herbivores, particularly in plants that evolved the ability to accumulate large quantities of Si. Very rapid herbivore-induced Si accumulation has recently been demonstrated, but the level of protection against herbivory this affords plants remains unknown. We aimed to investigate how quickly Si-based resistance to herbivory can be achieved in plants previously unexposed to Si.

Method

Brachypodium distachyon, a model Si hyperaccumulating grass, was exposed to the chewing insect herbivore, *Helicoverpa armigera,* and grown hydroponically under three conditions: supplied Si over 34 days (+Si), not supplied Si (-Si), or supplied Si only once herbivory began (-Si➔+Si). We evaluated the effectiveness of each Si treatment at reducing herbivore performance and measured Si-based defences and phenolics (another form of defence often reduced by Si) across three time points within 72 hr.

Results

Although Si concentrations remained lower, within 72 hr of exposure to Si, -Si➔+Si plants were as resistant to herbivory as +Si plants. Both +Si and -Si➔+Si treatments reduced herbivore growth and damage to plants, and increased mandible wear compared to -Si. After 6 hr, herbivory increased Si cell density in -Si➔+Si plants, and within 24 hr, -Si➔+Si plants reached similar Si cell densities to +Si plants, although decreased phenolics only occurred in +Si plants.

Conclusion

We demonstrate that plants with only short-term exposure to Si can rapidly accumulate Si-based anti-herbivore defences in a way that reduces herbivore fitness, performance, and damage to plants. Further, the extent of resistance achieved by short-term Si exposure is nearly identical to that of plants with long-term Si exposure. These findings provide novel insights regarding the temporal dynamics of Si-based plant defences and highlight that Si-mediated resistance to herbivory can occur much faster than previously envisaged.

KEYWORDS

Insect herbivory, plant defence, silica cells, silicon

Microbial involvement in insect-plant interactions

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Insect-plant interactions are dynamic events where both parties actively respond to the characteristics and activities of the other. Insects respond to plant characteristics such as visual and chemical cues and plants respond to physical and chemical aspects of insect presence. These processes involve various phenotypic changes and these have consequences for other members of the community. For instance, insect herbivory leads to the induction of plant defences that influence other herbivorous insects as well as carnivorous insects that feed on the herbivores. Among these carnivorous insects are parasitoids that oviposit in the herbivores, leading to the death of the herbivores. Experimental studies of insect-plant interactions have long focussed on the individual level, expanding from one plant and one insect to one plant and multiple insects at different trophic levels. This has resulted in important insights into the complexity of the dynamics of insect-plant interactions.

All macroorganisms harbour a community of microbes. Microbes play an ever more prominent role in the studies of insect-plant interactions. This relates to microbes that are associated with plants, herbivorous insects as well as carnivorous insects. Microbes such as endosymbionts may live in close association with insects, but there is also a dynamic microbial community associated with plants and insects. In this contribution I will highlight how microbes associated with plants, herbivores and carnivores influence insect-plant interactions or vice versa, relating to individual microbes as well as microbial communities. This relates to individual interactions that have consequences for population dynamics. This contribution will provide an outlook to the future of insect-plant-microbe interactions from individuals to populations.

Implications of switching invertebrate microbiomes on interactions with plants

The research topic of this discussion session will be the implications of switching microbiomes in invertebrates on their interaction with plants. In particular: 1) What are the environmental & biosecurity implications when introduced invertebrates exchange their associated microbes with native invertebrates in the novel environment; and 2) How do mechanisms like metabolism and other interactions with the environment alter when microbiomes of invertebrates switch and when newly acquired functional genes/gene-sets are expressed? We will use the intentional introduction of biological control agents as a model system and focus particularly on genes that have biosecurity implications, e.g. by changing the invertebrates' resistance to phytotoxins or by changing their diet. The topic will be relevant to microbiome, entomology and biosecurity researchers that are interested in synergising their expertise.

We invite you to participate in this discussion to form an international writing collaboration. The aim of this collaboration is to publish a synthesis paper, outlining a globally relevant research agenda for the next 5-10 years. Manaaki Whenua – Landcare Research (pronounced 'Mana'aki Phenua') is one of eight Crown Research Institutes in New Zealand. Our mandate is to drive innovation in the management of terrestrial biodiversity and land resources. We have extensive experience in biocontrol, biodiversity and biosecurity research and collaborate widely with other research organisations in New Zealand and around the world. Our team consists of experienced and early-career researchers with expertise in invertebrate, invasion, microbial and molecular ecology, biosecurity, biocontrol, genomics and evolution (Simon Fowler, Quentin Paynter, Thomas Buckley, Claudia Lange-ECR, Eva Biggs-ECR and Manpreet Dhami-ECR).

The discussion session will be an opportunity for researchers with similar and complimentary interests to connect. We will first introduce our team and present our idea for the synthesis paper. There will be opportunities during the presentation to vote on specific topics for the following discussion. Any questions will then be answered, and the selected topics be discussed with all participants. The goal of the session is to create a dedicated working group tasked with the synthesis of existing concepts in the field and development of a novel research agenda to advance this field. We anticipate that this international group will lead to high impact publications, exchange of mentorship for emerging researchers and new research networks.

AgriVectors: a community resource for arthropod vectors and agricultural pathosystems

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Arthropod vectors of pathogens cause enormous economic losses and are a fundamental challenge for sustainable food production, yet agricultural pathosystems remain an under-served area of research. A more effective strategy to fight plant diseases and their vectors, data pertaining to disease systems needs to be consolidated, made searchable with easy access for data mining. Thus, we created the AgriVectors™ platform as an open access and comprehensive resource for growers, researchers and industry working on plant pathogens and pathosystems spread by arthropod vectors.

Current resources include the Asian citrus psyllid, the potato psyllid and the bacterial pathogens they transmit to citrus and Solanaceous plants. Expansion to other important hemipteran vectors (whiteflies, leafhoppers, planthoppers, scale, mealybugs etc.) along with thrips and mites is planned. The open access portal connects established public repositories with pathosystem-specific data repositories. The portal is based on the Citrusgreening.org (https://citrusgreening.org/) community resource that was developed as a model for systems biology of tritrophic disease complexes. A demonstration will be provided of the resources for users with a short tutorial for attendees. Topics covered will include ’How to set up user accounts’ (Free) and ‘How to start’ using the digital resources to advance your research.

We would like to interact with the research communities working on pathogens and pests of vegetables, fruit trees/vineyards, and ornamentals in Europe. The AgriVectors system will provide tools to enable technologies such as non-transgenic RNAi, and CRISPR, with information on screening bioassays, etc. to leverage current and emerging knowledge across multiple disciplines. The platform also includes private and unpublished data, using where needed for restricted access.

The AgriVectors portal will extend this model beyond gene-centric omics data to the broader *System Biology of Pathosystem-wide* information, with integrated pest management, behavioral, plant health, soil health and climate data to incorporate rapid phenotyping information from research trials, building a foundation on which more effective solutions can be identified and developed to control plant diseases. We will create a follow up survey for all attendees to get feedback and plan for future user meetings.

Optimal foraging at small scales: Explaining the intra-inflorescence foraging strategy of a pollinivorous insect

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Animals have evolved foraging strategies to select food sources that maximise their fitness. In carnivores as well as in herbivores, these strategies have been mostly studied at the interspecific scale, i.e. when animals are confronted to food sources of different species. In herbivores, although specific within-plant patterns of resource exploitation, at the intra-individual scale, have been reported, it remains scarcely described and their determinants poorly understood.

Here, we describe and try to decipher the determinants of the foraging pattern of the pollen beetle (*Brassicogethes aeneus*), a pollinivorous insect that is a pest of oilseed rape (*Brassica napus*). This insect feeds from flowers for almost all of its life cycle, except for a couple of weeks preceding blossoming. During this period, only flower buds are available and the insect destroys them to feed from the pollen they contain, causing serious yield losses. We found that during this critical period, pollen beetles exhibit a stereotypic intra-inflorescence feeding pattern that depends on flower bud maturity.

To explain this pattern, we first deciphered the selective pressures driving pollen beetles' feeding behaviour. Since flowers are the natural food source of this insect, we used the preference for flowers over flower buds to achieve that goal.

Our results reveal that pollen beetles' feeding behaviour does not seem to be driven by specialised metabolites or an attempt to reach an optimal nutrient balance, but rather by a process of maximisation of total macronutrient intake. In a second time, we tried to explain the intra-inflorescence foraging pattern though the lens of total macronutrient intake maximisation. We found that results were fully consistent with this hypothesis. Thus, even at small scale, nutritional challenges caused by resource heterogeneity can influence foraging responses of animals. The present study represents new insights into the forces responsible for the evolution of feeding behaviours.

Keywords: feeding behaviour, evolutionary forces, specialised metabolites, macronutrient balance, macronutrient intake

Silicon-mediated herbivore defence in a pasture grass under pre-industrial and Anthropocene levels of CO_2

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Abstract

Silicon (Si) is a ubiquitous in soil and a beneficial element for plants, particularly under environmental stresses. Si helps plants to alleviate multiple biotic and abiotic stresses. Si mitigates both biotic and abiotic stresses by enhancing plant physical and biochemical defences (e.g antioxidant enzymes (AE)). Si application increases AE system which helps to reduce oxidative stress caused by reactive oxygen species (ROS). Atmospheric CO_2 levels are known to affect grass Si concentrations which, in turn, can alter herbivore performance. Pre-industrial CO_2 and Anthropocene CO_2 causes increase and decrease Si accumulation in grasses, respectively. However, it is unknown how increased and decreased Si accumulation under pre-industrial CO_2 and Anthropocene CO_2, respectively, can affect AE activities and the consequences for insect herbivory. Using tall fescue (*Festuca arundinacea*) grass and insect herbivore*, Helicoverpa armigera*, we examined the effect of Si treatments on AE activities of plants grown under three CO_2 levels (200, 410, and 640 ppm) and the consequences for insect herbivory. Regardless of CO_2 conditions, Si treatments increased antioxidant defence ability, which may help to reduce herbivore-induced ROS oxidative stress. Both Si supplementation and eCO_2 reduced herbivore relative growth rate (RGR). Si treatment declined herbivore RGR by (-65% and -38%) under reduced CO_2 and elevated CO_2, respectively. Si treatments also decreased frass produced. This study suggested that Si application may increase grass biochemical defences system against insect herbivore irrespective of atmospheric CO_2 levels.

Keywords: Antioxidant enzymes, biochemical defence, carbon dioxide, insect herbivore, silicon

Silicon defences and *Epichloë*- symbiosis in tall fescue diminish feeding efficiency and immunity of an insect herbivore

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Session: The ecology of plant-insect interactions

Abstract

1. Many grasses lack the diversity of defensive secondary metabolites found in other plant families, and thus rely on physical defences and symbiotic associations for defence against herbivores. In particular, silicon (Si) is a structural defence that can account for as much as 10% of dry mass. Furthermore, many temperate grasses associate asymptomatically with *Epichloë*-endophytes that also facilitate herbivore resistance *via* defensive-alkaloid production. Recent evidence suggest that *Epichloë*-symbionts increase Si accumulation in their host grass, but it is unknown how this process affects specific Si-defences in the leaf surface and subsequently, the impacts on feeding efficiency or immunity to potential infection of herbivores.

2-3. To address this knowledge gap, we grew tall fescue (*Festuca arundinacea*) hydroponically, with and without Si, in the absence or presence of AR584-*Epichloë*-strain, and exposed plants to *Helicoverpa armigera* in both *in-situ* (intact plants) and *ex-situ* (excised leaves) feeding trials. Subsequently feeding efficiency and immunity of caterpillars was subsequently measured.

4. We report that endophyte and Si-supply both increased plant growth by 15%, foliar Si by 127%, and leaf silica cell density by 46%. Moreover, herbivory by *H. armigera* in combination with endophyte and Si-supply further induced levels of Si by 143%, and silica cell density by 110%. Despite the observed endophyte-mediated increases in Si, only Si-supply reduced *H. armigera* growth by 87%, feeding efficiency indices by at least 30%, and mandibular wear by 16% in the *ex-situ* feeding trial. Moreover, Si had negligible effects on alkaloids produced by endophytes. Conversely, when *H. armigera* immune responses were evaluated, we found that both Si and endophytes negatively affected insect's immunity, increasing, in theory, their susceptibility to infection or parasitism (*i.e.,* biocontrol).

5. This study provides novel evidence that Si defences and endophytes can work compatibly to increase grass resistance to herbivorous chewing insects.

Keywords: Silicon (Si), *Epichloë*-endophytes, alkaloids, *Festuca arundinacea*, insect immunity, *Helicoverpa armigera.*

Identification of carboxylesterases in Arabidopsis leaves that catabolize the herbivore-induced plant volatile (*Z*)-3-hexenyl acetate.

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The herbivore-induced plant volatile (*Z*)-3-hexenyl acetate (Z3-6Ac) is known to prime plant defenses against insect herbivore attack, yet how plants perceive Z3-6Ac is not well understood. Here, we show that the model plant *Arabidopsis thaliana* readily absorbs and catabolizes exogenous Z3-6Ac, and propose that metabolic processes could serve to regulate Z3-6Ac perception and downstream signal transduction. We found that shortly after exposing Arabidopsis plants to synthetic Z3-6Ac, intact leaves accumulated the related volatile (*Z*)-3-hexenol and its corresponding glucoside, (*Z*)-3-hexenyl-β-glucopyranoside. Further investigation revealed that one or more enzymes present in Arabidopsis leaves, as well as in leaves taken from various other plant species, catalyze the conversion of Z3-6Ac to (*Z*)-3-hexenol. Two such enzymes were purified from Arabidopsis crude leaf extracts and identified by peptide sequencing as carboxylesterases, thereby allowing us to isolate an Arabidopsis mutant line with diminished Z3-6Ac esterase activity. Studies are now underway to discern whether carboxylesterase knock-out mutants exhibit differential responses to Z3-6Ac exposure and herbivore feeding relative to wild-type plants.

Keywords: Arabidopsis; defense priming; volatiles; plant-herbivore interactions; plant-plant interactions.

Intraspecific plant variation and non-host herbivores affect parasitoid patch choice and residence time through changes in apparency of upwind volatile information

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Objective

Parasitoids need to find their hosts in patchy environments that differ in quality. To maximize foraging efficiency, parasitoids use volatile information of the plants on which their hosts feed. The blend of volatiles emitted is affected by herbivore species and genetic variation in plants. However, how parasitoids deal with variation in plant volatiles induced by host or non-host herbivores on various plant genotypes in a plant stand is unclear.

Method

In a wind tunnel, we examined foraging behavior of the parasitoid *Cotesia glomerata* in mixtures of white cabbage cultivars that are induced by host (*Pieris brassicae*) and non-host herbivores (*Mamestra brassicae* or *Delia radicum*). We specifically studied the efficiency of parasitoids in locating a host infested plant when having to pass three other plants that vary in volatile emission by cultivar and herbivore identity.

Results

We show that foraging decisions of *Cotesia glomerata* are affected by the apparency of upwind host patches. We found that parasitoids flew over the first two plants more often when the last plant was a host infested attractive cultivar Christmas Drumhead and the first three plants were the less attractive cultivar Rivera, regardless of the presence of host (*Pieris brassicae*) or non-host (*Delia radicum* & *Mamestra brassicae*) herbivores. Furthermore, parasitoids spend more time on the first three plants if these were infested with host or non-host caterpillars, and this effect was stronger when the first three plants were of the attractive Christmas Drumhead cultivar.

Conclusion

Our results suggest that parasitoids may more easily locate host herbivores on plant genotypes with more apparent volatile information. However, host location efficiency is affected by contrast with other plant volatile plumes derived from genotypic variation in plants and induction of volatiles by non-host herbivores. Apparency of information on upwind patches influences patch residence time and patch choice.

KEYWORDS
Marginal Value Theorem – Foraging behavior – Parasitoid – HIPV – *Cotesia glomerata*

ABSTRACT TITLE

Beating the bugs in the cranberry bogs – host plant resistance in cranberry

AFFILIATIONS

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ABSTRACT TEXT

Objective

Insect pests are problematic in the production of cranberry (*Vaccinium macrocarpon* Ait., Ericaceae). Spraying chemical pesticides is common practice, but could also affect beneficial insects, such as pollinators and natural enemies. Therefore, we studied the defensive mechanisms that cranberry plants themselves use to combat insect pests, focusing on gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Erebidae), an occasional but devastating pest of cranberry in the United States.

Method

Direct and indirect defenses of several wild and hybrid varieties were studied – cranberry is a recent crop, domesticated ca. 200 years ago, and wild selections are still commonly grown. Domestication and artificial selection for increased yield may have had a cost for other potentially useful traits, such as host plant resistance. In a greenhouse setting, gypsy moth performance, phytohormone and phenolic production, defense-related gene expression, and volatile emission were assessed.

Results

Gypsy moth feeding increased the expression of two genes associated with terpene biosynthesis, as well as the emission of herbivore-induced plant volatiles. There were differences between varieties in the emission of mono- and sesquiterpenes, but not gene expression, and there were no distinct differences between wild selections and hybrids.

Conclusion

Cranberry varieties differ in their insect resistance qualities, but there were no distinct differences between wild selections and hybrids, indicating that cranberry, unlike other crops, was selected not only for higher yield, but also for increased host plant resistance. Elucidating mechanisms of resistance in cranberry will help improve the development of more sustainable pest management practices in existing varieties, as well as development of novel varieties with improved resistance qualities.

KEYWORDS
Host plant resistance, chemical ecology, *Vaccinium macrocarpon*, gypsy moth

Geranium macrorrhizum, a potential novel companion plant to control aphid populations

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The combination of a companion plant with a cultivated plant is considered as an interesting strategy to reduce pest pressure and hence, the use of pesticides. Although several plants from the *Alliaceae* and *Lamiaceae families* are known to be efficient companion plants against aphid pests, only a few plants of the *Geraniaceae* family have been studied so far. The aim of this work was to investigate the potential effects of *Geranium macrorrhizum* as a companion plant on the colonization of sweet pepper *(Capsicum annuum, Solanaceae*) by the green peach aphid (*Myzus persicae)*. Aphid's feeding behavior and life history traits were assessed on sweet pepper using EPG (ElectroPenetroGraphy) and clip-cage laboratory bioassays, respectively. They were compared in absence or presence of *G. macrorrhizum* whose leaves had been mechanically stimulated or not.
The composition of VOCs from *G. macrorrhizum* leaves was analyzed using SPME technic followed by GC-MS. This study revealed that *G. macrorrhizum* as a companion plant negatively impacted the feeding behavior, fecundity and survival rate of *M. persicae* on sweet pepper. The effects were exacerbated when *G. macrorrhizum* leaves were mechanically stimulated. This could be due to the greater amount of the main VOCs Germacrone and Elemenone emitted by *G. macrorrhizum* following mechanical stimulation. Our results bring new insights into the use of novel companion plants to regulate aphid pest populations.

KEYWORDS: Companion planting, VOC, aphid colonisation, *Geraniaceae*, behaviour

What goes in must come out? The metabolic profile of plants and caterpillars, frass, and adults of *Asota* (Erebidae: Aganainae) feeding on *Ficus* (Moraceae) in New Guinea

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Insect herbivores have evolved a broad spectrum of adaptations in response to the diversity of chemical defences employed by plants. Here we investigate adaptations in one such plant–insect system in detail, testing the hypothesis that two species from the moth genus *Asota* sequester alkaloids from their *Ficus* hosts as caterpillars and partition them across body parts as adults. As our two focal caterpillar species are restricted to one of three chemically distinct *Ficus* species, we also test whether or not these specialised interactions lead to similar alkaloid profiles in both *Asota* species.

We reared the two *Asota* species on three species of *Ficus* in natural conditions and analysed alkaloid profiles of leaf, frass, caterpillar, and adult moth samples using UHPLC–MS/MS analyses. We then tested if the alkaloid profiles differed among individual sample types and explored the fate of individual leaf alkaloids when ingested by the insects.

We identified 43 alkaloids in our samples. Leaf alkaloids had various fates. Some were retained in caterpillar tissue while others were excreted in frass or found in certain body parts of adult moths. We also found two apparently novel indole alkaloids present in both caterpillar and adult tissue but not in leaves or frass. We hypothesize that these metabolites were likely synthesised *de novo* by the moths or their associated microbiome. Overall, alkaloids that were unique or restricted to insect tissue were shared across moth species despite feeding on different hosts. This indicates that a limited number of plant compounds have a direct ecological function that is conserved among the studied species.

Our results help illustrate how specialist herbivores cope with their host plants' chemical defences and sometimes even use such defences for their own benefit. Furthermore, we provide a new example of pharmacophagy in an ecologically and medically relevant insect genus.

Keywords: metabolomics, alkaloids, sequestration, de novo synthesis, tiger moth

Strong impact of leaf flush on oaks secondary chemistry and on their primary consumers

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ABSTRACT

Temperature influences plant budburst, the production of new shoots and leaves, and the end of its activity. Trees that produce multiple leaf flushes present a combination of leaves that differ in secondary chemistry, and that change through the growing season. Thus, herbivores and pathogens face leaves with high and low content in secondary chemistry during each season. We assessed the impact of oak leaf flush on secondary chemistry, on herbivory and on pathogen infection in European oak forests by sampling 20 populations in 3 parts of the growing season. As well, we investigated the mediation of and secondary chemistry on the impact of leaf flush on herbivory and pathogen infection. Despite the variation on secondary chemistry among leaf flushes during the growing season, the first leaf flush generally had lower percentages of flavonoids and tannins than the subsequent leaf flushes, while lignins followed the opposite pattern. The accumulation of herbivory gradually decreased from the first to the third leaf flush through the growing season. The first leaf flush exhibited a higher resistance to pathogen infection, while the subsequent flushes where susceptible to infections by oak powdery mildew. The impact of secondary chemistry on herbivory and pathogen infection strongly differed among leaf flushes and seasons. Importantly, the role of leaf flush on herbivory and pathogen infection, as well as its connection with plant secondary chemistry is likely to play a major role on interactions between primary producers and primary consumers in oak food webs.

Keywords: herbivory, leaf flush, pathogen infection, *Quercus robur*, secondary chemistry

Silicon augments plant tolerance to insect herbivory

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Objective

We present the effects of silicon (Si), a beneficial plant nutrient, on plant resistance and tolerance to insect herbivory.

Method

We grew the model grass, *Brachypodium distachyon*, hydroponically with (+Si) or without Si (−Si) and investigated their resistance and tolerance to a global insect herbivore, the cotton bollworm, *Helicoverpa armigera*. Half of the plants of each Si treatment were exposed to a single *H. armigera* larva for seven days and subsequently, we measured leaf gas exchange rates, non-structural carbohydrates in leaf and root, plant biomass and larval growth and feeding. Plant measurements were analysed using two-way analysis of variance (ANOVA) tests, incorporating Si and herbivory as fixed factors. Larval growth and feeding were analysed using one-way ANOVA tests.

Results

Silicon and herbivory reduced leaf net photosynthesis. Silicon also reduced stomatal conductance and transpiration rate whereas herbivory increased both. The concentrations of leaf soluble sugars (% dry weight) were higher in insect-fed, +Si plants compared to insect-fed, −Si plants. Moreover, Si increased the concentrations of root soluble sugars irrespective of herbivory.

Herbivory reduced the concentrations of leaf starch regardless of Si treatment while root starch concentrations were unaffected by herbivory or Si treatment. Furthermore, plant compensatory growth following herbivory was higher in +Si plants than −Si plants and insect feeding induced Si accumulation in leaves. Besides, Si reduced larval growth and consumption.

Conclusion

Our results suggest that Si not only underpins plant resistance but also augments plant tolerance to insect herbivory possibly via enhanced concentrations of plant soluble sugars.

Keywords: silicon induction, gas exchange, non-structural carbohydrates, compensatory growth, plant resistance,

ABSTRACT TITLE

Changing tomato trichome phenotypes does not influence the plant metabolome induced in response to thrips and spider mites

AFFILIATIONS

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ABSTRACT TEXT

Objective
Herbivores encounter a plethora of plant defence mechanisms that hamper their feeding activities. A first line of structural defences in many plants are trichomes, outgrowths from the epidermis that produce, store and release toxic metabolites. Besides, herbivory induces large transcriptomic and metabolomic changes in plants associated with elevated defences. Furthermore, trichome density and their synthesis of feeding deterrents can increase upon attack, offering an ideal framework to explore the link between constitutive and inducible plant defences. Here, we evaluated how modifying the plant trichome phenotype influences plant responses induced by herbivores.

Method
We used two genetically characterized tomato trichome mutants, called hairless (*hl*) and hairs absent (*Hair*), to evaluate whether a decreased density of glandular trichomes renders the plants more vulnerable to two herbivorous pests: the two-spotted spider mite (*Tetranychus urticae*) and the western flower thrips (*Frankliniella occidentalis*). Subsequently, we conducted untargeted metabolomics on leaves of mutant and wild-type plants with or without these herbivores.

Results
We observed that tomato type VI trichomes are highly effective against the two-spotted spider mite but to a lower extent against the western flower thrips. The untargeted metabolomics analysis revealed that exposure to herbivores, but not the plant genotype, had a significant effect on the overall tomato leaf metabolome. In particular, herbivores modulated the accumulation of fatty acids, dipeptides, compounds related to the metabolism of amino acids, and others previously described in response to pathogens.

Conclusion
While trichome composition can have a profound effect on the level of resistance of tomato plants against herbivores this seems independent from the plant inducible metabolome. This research expands our understanding on the interdependence between constitutive and induced defences in an important model plant like tomato.

KEYWORDS

Trichomes, herbivores, untargeted metabolomics, induced plant defences, tomato mutants.

Plant-to-plant communication in response to insect herbivory is not specific to genetic relatedness between emitter and receiver *Solanum tuberosum* plants

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Recent studies in wild plants have underscored the specificity of plant-plant signalling via volatile organic compounds (VOCs) with respect to plant genetic relatedness, where more closely related species are expected to exhibit stronger signalling. However, a mechanistic understanding of variation in volatile blends underlying this plant genetic specificity in signalling is lacking, and would be especially impactful for pest control in cultivated plants. Here, we investigated whether airborne signalling in cultivated potato (*Solanum tuberosum*) plants in response to leaf herbivory by *Spodoptera exigua* was contingent on plant genetic relatedness, and further investigated the VOCs underlying any such plant genetic-based specificity. We carried out a greenhouse 2 by 2 factorial experiment using 15 commercial varieties of potato for which we placed pairs of plants (i.e. emitters and receivers) of the same vs. different variety in plastic cages and for half of the pairs the emitter was damaged by *S. exigua* whereas for the other half the emitter was left intact (controls). Three days later, we measured quantitative and qualitative changes in VOC emissions for emitter plants, and placed *S. exigua* larvae on receivers to test for effects on the amount of leaf damage and larval weight gain for 48 h. Results showed that the percentage of leaf area consumed by *S. exigua* larvae and larval weight gain on receiver plants exposed to damaged emitters were, on average, significantly lower compared to leaf damage and weight gain of larvae on receivers exposed to control emitters, i.e. plant signalling boosted resistance in intact receivers. However, we found that plant signalling effects did not depend on emitter and receiver genetic relatedness, suggesting no genetic-based specificity. Overall, this study demonstrates that airborne signalling between potato plants impacts leaf herbivory irrespective of plant genetic-based specificity, with potentially important implications for sustainable pest control in this crop.

KEYWORDS
Plant varieties, plant communication, *Solanum tuberosum*, *Spodoptera exigua*, volatile organic compounds.

Pea aphid (*Acyrthosiphon pisum*) host races differently influence phloem protein (forisome) based phloem-sealing defenses

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To prevent loss of phloem sap through damage or phloem feeding insects, plants have developed different sieve element (SE) occlusion mechanisms. Two main mechanisms are known to date: callose mediated and phloem (P-) protein mediated mechanisms. Forisomes, P-proteins unique to papilionoid legumes such as bean (*Vicia faba*), pea (*Pisum sativum*), clover (*Trifolium pratense*) or alfalfa (*Medicago sativa*), reversibly change their conformation (i.e. disperse) after Ca^{2+} influx into SEs. This leads to occlusion of SEs and stops the loss of phloem sap. Phloem feeding insects that are not native to bean such as the green peach aphid (*Myzus persicae*) have been shown to trigger forisome dispersion, whereas the native pea aphid (*Acyrthosiphon pisum*) evaded this response.

However, the pea aphid is a complex of at least 15 genetically distinct, sympatric host races which are native to different host plants such as pea or clover but can all feed on bean, the universal host plant. This raises the question, whether different host races have developed different ways to manipulate forisome dispersion and whether they can prevent dispersion in response to a strong stimulus such as heat. Therefore, we investigated different host race – plant combinations. The different host races were not able to prevent heat triggered forisome dispersion in the universal host plant (bean) or in pea. However, feeding of the clover host race on clover suppressed forisome dispersion after heat stimulation, whereas other host races were not able to do this. This shows that the different host races have developed different mechanisms to not only circumvent triggering forisome dispersion but also to prevent dispersion in response to strong stimuli.

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Plants mediate interactions among insect herbivore via induced defences. These effects are predicted to be contingent on the herbivore's feeding guild, whereby prior feeding by insects should negatively impact subsequently feeding insects of the same guild (induced resistance) but positively influence insects of a different guild (induced susceptibility) due to interference between plant signaling pathways differentially associated to each insect. Here, we investigated the effects of prior feeding by leaf-chewing caterpillars (*Spodoptera frugiperda*) and sap-sucking aphids (*Aphis gossypii*) in wild cotton (*Gossypium hirsutum*) plants on subsequently feeding by caterpillars (*S. frugiperda*) and on plant chemical defences. In a greenhouse experiment, we had cotton plants either undamaged, or previously exposed to caterpillar or aphid feeding, and then we placed caterpillars to assess their performance and their damage on plants. Leaves were also collected on a separate set of experimental plants to assess the concentration of phenolic compounds. The amount of leaf area consumed by caterpillars on plants previously attacked by aphids and caterpillars was significantly reduced relative to those feeding on controls (72% and 77% less leaf area, respectively). Likewise, caterpillar weight gain and survival were significantly lower on plants with prior aphid (59% weight gain decrease) and caterpillars (46% survival decrease) feeding respectively, relative to controls. These caterpillar performance variables did not differ significantly between aphid and caterpillar treatments. In addition, we found that aphid and caterpillar feeding drove significant 77% and 62% increases, respectively in flavonoids, and 90% and 62% increases, respectively in lignins relative to controls. Flavonoid and lignin levels did not differ between aphid and caterpillar treatments. Overall, these findings provide no support for induced susceptibility to caterpillars due to prior aphid feeding. Instead, they indicate that prior aphid and caterpillar feeding similarly induce cotton defences and cause equivalent levels of induced resistance against subsequently feeding caterpillars.

ABSTRACT TITLE

What is role of sesquiterpenes in behavior of *Plutella xylostella* (Lepidoptera: Plutellidae)?

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Assistant Prof. James Blande: original idea for work, concept planning and supervision

ABSTRACT TEXT

Objective

Sesquiterpenes are herbivore induced plant volatiles (HIPVs) but their role in plant-insect interactions remains still poorly understood. Li & Blande (2015) find that sesquiterpenes ((E,E)-α-farnesene, α-selinene and β-elemene) emitted from neighboring plant, adsorbed to leave surfaces of receiver plant and made it more susceptible for oviposition of *Plutella xylostella*. This finding of passive plant-plant interaction suggests that sesquiterpenes may have an important ecological role by regulating host selection and oviposition behavior of *Plutella xylostella*. With this hypothesis, we conduct series of laboratory experiments to understand mechanisms of sesquiterpenes in plant-insect interactions. Aim of this project is to apply this information in development of environmentally sound pest control strategies for brassicaceous crops.

Method

Main methods are: 1) VOC-profile characterization from selection of *Brassica*-plant genera to identify levels of sesquiterpene emissions. 2) Oviposition tests for *P. xylostella* between high and low sesquiterpene emitter plants. 3) Two-choice olfactometer tests between *Brassica* species used in oviposition tests and with pure sesquiterpenes.

Results

We have targeted a landrace turnip (*Brassica rapa* var. *Rapa*) to be a strong emitter of sesquiterpene (E,E)-α-farnesene compared to white cabbage (*Brassica oleacera* var. *capitata*) and broccoli (*Brassica oleacera* var. Italica). Turnip was highly attractive to *P. xylostella* in our oviposition and olfactometry tests. Olfactometry tests, conducted in June and July 2021, will confirm role of (E,E)-α-farnesene in female *P. xylostella* behavior based on olfaction signals.

Conclusion

We have found interesting results showing possible connection between high sesquiterpene emission of turnip and its high attractiveness for *Plutella xylostella*. It is crucial to separate the role of sesquiterpenes from other factors, in behavior of *P. xylostella*. To answer this, we test their impact as a pure compound and as part of plant volatile emission blend.

KEYWORDS
Plutella xylostella, sesquiterpenes, *Brassica oleacera*, *Brassica rapa*, (E,E)-α-farnesene

ABSTRACT TITLE:
Speciation in a 3-species fig complex along a tropical elevation gradient: gene flow and the role of the pollinating wasps

AFFILIATIONS

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ABSTRACT TEXT

Objective:
We present evidence for gene flow among parapatric montane fig species and discuss the role of the pollinating wasps in the speciation history of the complex. We focus on three species in a monophyletic complex endemic to Papua New Guinea with the aim of eventually documenting the genomics of diversification from a single lowland ancestor.

Method
We used Single-Nucleotide Polymorphism (SNP) data from two populations from each of three species. We studied, *Ficus microdictya, F. umbrae* and *F. itoana*, each occupying high, mid and low elevations respectively. Each species is primarily pollinated by a specific wasp of the genus *Ceratosolen* (1-1 mutualism). To evaluate potential signs of introgression, we performed an ABBA-BABA test using the closely related *F. trichocerasa* as the outgroup: (((*F.microdictya, F.umbrae*), *F.itoana*), *F.trichocerasa*). We additionally performed admixture analysis.

Results
The ABBA-BABA test showed significant signs of introgression between the mid-elevation species *F. umbrae* and low-elevation species *F. itoana* (D=0.287, Z=7.526, p<0.001), with relatively low levels of admixture (F=0.152). Admixture analysis suggested four groups as the optimal number of clusters for the three fig species, with the cross-validation error (CVE) for the 4-K grouping being only slightly lower than the 3-K CVE.

Conclusion

Speciation in figs is intertwined with the host preferences of the corresponding mutualistic pollinating wasps. Gene flow between parapatric species of figs indicate that hybridization has occurred despite the assumed host specificity of the pollinators. It is yet to be explored whether this gene flow is historical or ongoing, as well as which is the dominant direction of the gene flow. Following steps will clarify the mechanisms of speciation in wasps and the role of alternative factors, such as the microbial agent *Wolbachia* hosted in wasps, in shaping the coevolutionary history of the fig-wasp system.

KEYWORDS
figs – evolution – genomics – gene flow

Improved in-crop monitoring and use of trap cropping as novel approaches to the integrated pest management of aphid BYDV vectors in winter cereals

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Barley yellow dwarf virus (BYDV) is one of the most widespread and economically important plant viruses. In the UK, two aphid species are primarily responsible for transmitting BYDV: the bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus) and the English grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). The management of BYDV is becoming increasingly difficult due to decreased pesticide availability, emergence of insecticide resistance in aphid vectors and climate change. Integrated pest management (IPM) reduces the reliance on synthetic pesticides by using monitoring to inform the use of controls, preventing pest problems wherever possible and using a diverse range of controls. This project tests whether the use of wheat varieties that are more attractive to aphid BYDV vectors are effective as trap crops in reducing the numbers of aphids entering the main crop. In addition, the chemical cues used by cereal aphids in host plant selection will be studied in order to develop better monitoring tools. Recent work at Harper Adams University investigating the landing behaviour of the English grain aphid reported that significantly more aphids landed on Maris Huntsman, a heritage wheat variety, over modern AHDB Recommended Lists varieties, which shows its potential to be used as a trap crop. We are currently investigating the landing behaviour of the BYDV infected and non-infected bird cherry-oat and English grain aphid populations on 19 winter wheat varieties including Maris Huntsman and the BYDV resistant variety Wolverine. This is being done by releasing 60 winged aphids from the centre of cages, equidistant from each wheat variety placed in a circle around the release point. The landing behaviour of alates is being monitored at regular intervals after release. With these results, we aim to identify varieties which are more attractive to aphids and investigate the olfactory-basis for selection.

ABSTRACT TITLE

Genome-wide gene birth-death dynamics are associated with diet breadth variation in Lepidoptera

AFFILIATIONS

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ABSTRACT TEXT

Objective

Both the duplication and loss of genes have been shown to facilitate the evolution of novel, adaptive traits. Thus, the study of gene birth-death dynamics (GBDD) has the potential to reveal gene families that have critically shaped morphological, behavioral or physiological variation across a lineage. Here, we used whole genomes of 30 species to investigate patterns of GBDD among the Lepidoptera and to link these patterns with either specialist or generalist feeding strategies.

Method

We investigated gene GBDD within the higher Lepidoptera (Ditrysia) by first generating *de novo* annotations for publicly available reference genomes and then using the annotations as input for the tool CAFE. With CAFE, we both estimated gene family expansion and contraction rates across Ditrysia and identified significant GBDD at the crown node of Papilionoidea (i.e. at the node butterflies originated). The results from our analyses in CAFE were paired with hostplant data in a single, multilevel Bayesian model that enabled us to detect significant differences in gene family size along a specialist-generalist feeding axis.

Results

We discovered that the sizes of several important gene families (e.g., those associated with pesticide resistance, xenobiotic detoxification, and/or protein digestion) are significantly correlated with diet breadth. We also found 10 functional gene families showing significant GBDD at the Papilionoidea node, including digestive proteins and odorant receptors.

Conclusion

Our findings show significant genomic differences between generalist and specialist Lepidopteran taxa that warrant further exploration. Furthermore, we demonstrate the value of pairing GBDD analyses with life history data to identify repeated patterns of evolution in distantly related clades.

KEYWORDS

comparative genomics, Lepidoptera, gene birth-death dynamics, diet breadth

Estimating the attraction range of pheromone traps to optimise biocontrol - using smallholder systems as case study

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Objective To effectively employ pheromone trapping, trap densities should be high enough to reduce the local male population. The objective of this presentation is to present in layman terms a statistical method that can estimate the attraction range of pheromone traps and the average insect population density. These estimates can subsequently be used to optimise trap densities in cases where pheromone traps are used for monitoring and biocontrol.

Method Our statistical method is based on the observation that pheromone traps that are placed closer to each other are more strongly competing for male insects than traps that are positioned further away from each other. We illustrate our method on a case study of smallholder farmers in Bangladesh that grow eggplants and that suffer from severe yield losses (30-90%) due to the eggplant shoot and fruit borer moth (*Leucinodes orbonalis*). In this setting a field experiment was performed with 4 and 24 traps that was for model fitting.

Results The statistical method could reproduce the observed patterns very well. The distance from the trap within 95% of the individuals are caught is 36 meters, and the average male moth density is 0.028 individuals/m^2. We show that a single farmer cannot effectively employ pheromone traps as the attraction range is larger than a typical field size. At least 9 traps (four farmers) should be placed at a distance of 10 meter to increase crop yields and outweigh the costs of installing and maintaining traps.

Conclusion The statistical method developed maybe a useful tool in biocontrol settings to optimise the densities of pheromone traps. Additionally, our case study convincingly shows that collaboration between smallholder farmers is needed to benefit from mass trapping by pheromone traps.

Attraction range, biocontrol, trap density, pheromones, traps

ABSTRACT TITLE

Assessing the risk to plant health from future *Agrilus* (Coleoptera: Buprestidae) invasions

AFFILIATIONS

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ABSTRACT TEXT

Objective

Agrilus is a genus of phytophagous jewel beetles. Some, like Emerald Ash Borer, have proved devastating to novel hosts in new areas. This raises the question of whether other seemingly innocuous *Agrilus* species could pose a threat outside their native range. We aim to assess the risk to the UK flora from future *Agrilus* invasions, i.e., to identify *Agrilus* species that pose the highest threat and plants most at risk.

Method

We compiled a dataset of known *Agrilus* hosts through a literature search and cross-referenced it with a list of flora from the British Isles for potential hosts. We also extracted phylogenetic data from the oneKP Initiative and pruned it to angiosperm family level (presenting 59.33% of all angiosperm families) to visualise the number of *Agrilus* species hosted per family.

Results

We have information for 308 *Agrilus* species that utilise 415 plant species (161 genera, 47 families) – mostly eudicot angiosperms. We observe some clustering of host families, indicating there may be phylogenetic signal in host range. Most host genera (57.23%) and species (67.98%) only host one *Agrilus* species, and most *Agrilus* are monophagous: 87.46% use one plant family, 74.31% one genus and 53.21% one species. However, some polyphagous species exploit distantly related families.

In the British Isles, there are nine recorded *Agrilus* species. However, there are 70 plant genera (which can host 189 *Agrilus*) and 109 species (which can host 75) that are known *Agrilus* hosts – some of which could be at risk from future invasions.

Conclusion

We identified known and potential UK *Agrilus* hosts that may be at risk from future invasions. There is indication of phylogenetic signal in host range, which will allow us to next perform phylogenetic analyses to determine which plants are more likely to become novel hosts and are thus most at risk.

KEYWORDS

invasive species, biological invasions, host range, novel interactions, phylogenetic signal

Title

A transcriptomic dig into plant defense against a root herbivore

Affiliations:

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Objective

During my talk, I will present results of an extensive RNAseq experiment in which we delved into plant defense against a root herbivore, and how this response is modulated by prior foliar herbivory.

Methods

We investigated the transcriptome of cabbage plants (*Brassica oleracea*) in response to cabbage root fly larvae (*Delia radicum*). Prior to root herbivore attack, leaves of these plants were exposed to herbivores belonging to two feeding guilds: leaf-chewing diamondback moth caterpillars (*Plutella xylostella*) or phloem-feeding cabbage aphids (*Brevicoryne brassicae*). We performed RNA-sequencing at 5 time points and extensively analysed this data. We used a *myb28* Crispr knockout *B. oleracea* line to confirm effects of aliphatic glucosinolates on cabbage root fly. We also performed a follow-up experiment using qPCR in which we focus on early defense signalling and priming.

Results

We show that *D. radicum* has a major impact on the cabbage root transcriptome, changing thousands of genes. Many of these genes are involved in commonly studied defense processes, while others are more surprising, perhaps due to differences between leaves and roots, or due to the specialist nature of this herbivore. Prior herbivory by caterpillars decreases survival of root herbivores. We show that plants respond faster to root herbivory when previously attacked by caterpillars, suggesting that defense priming plays a role in this plant-mediated interaction. Finally, we show that aliphatic glucosinolates confer resistance to this specialist root herbivore.

Conclusion

Plants heavily invest in plant defense against root herbivory. Prior herbivory by caterpillars leads to a faster root response, leading to better defense.

Keywords

Above-belowground interactions, defense priming, root herbivory, RNAseq, glucosinolates, mutant analysis

Accidental introduction of *Candidatus* Liberibacter europaeus into New Zealand Scotch broom via a weed biocontrol agent from the UK

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The bacterium, *Candidatus* Liberibacter europaeus (Leu) was reported in New Zealand (NZ) in 2011 in Scotch broom, *Cytisus scoparius* Link, and in its deliberately released biocontrol agent, the broom psyllid *Arytainilla spartiophila* Förster. We retrospectively assessed the risk of Leu being accidentally imported into NZ with broom psyllids in the 1990s, investigated seed transmission of Leu, and examined bacterial diversity in Leu-infected and non-infected Scotch broom.

We developed species-specific primers for Leu detection and conducted end-point and real-time PCR analyses for Leu in broom psyllids and potential host plants across NZ. The bacterial community in broom stems and seeds was investigated by 16S metabarcoding.

Importation methods for UK broom psyllids into NZ in the 1990s provided a likely pathway for inadvertent release of Leu in NZ. Leu in individual NZ Scotch broom plants was significantly associated with the presence of broom psyllids, and Leu was absent from plants at sites without psyllids. There was no evidence that Leu was present in NZ before the release of broom psyllids, and Leu from UK and NZ also had identical partial 16S sequences. Vertical transmission of Leu is possible as we detected Leu in F1 seeds. We are currently analysing bacterial diversity in Leu-infected and non-infected broom stems and seeds.

Leu was likely imported into NZ in deliberately released broom psyllids in the 1990s, a biosecurity breach that was largely unavoidable because detection methods for this unculturable organism were undeveloped at that time. It remains uncertain whether Leu is pathogenic in Scotch broom, or symptomless as in pears. Unpredictable impacts of *Ca*. Liberibacter spp. in plant species implies that new weed biocontrol agents will need to be free of such potential plant pathogens. In future, molecular characterisation of insect microbiomes could become essential for pre-release safety screening of biocontrol agents.

Candidatus Liberibacter europaeus; Scotch broom; *Cytisus scoparius*; *Arytainilla spartiophila*

Modulation of jasmonate pathways in rice (*Oryza sativa*) by the microbiota present in the oral secretions of the Lepidopteran pest *Spodoptera frugiperda*

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Spodoptera frugiperda (Lepidoptera: Noctuidae) is an insect pest native to America, which has recently spread throughout the world. Its polyphagous caterpillar attacks a wide variety of host plants, including some of great economic interest such as corn (*Zea mays*) or rice (*Oryza sativa*). These plants recognize herbivorous insects thanks to Herbivore Associated Molecular Patterns (HAMPS), such as β-glucosidase, fatty-acid-amino acid conjugates and inceptins, present in the oral secretions (OS) of the insect. These elicitor HAMPs will trigger the synthesis of defense molecules, in particular through the activation of derivatives of the jasmonate (JA) pathway. Our objectives are (i) to study the activation of the JA pathway in rice in response to herbivory, (ii) to evaluate the role of the insect gut microbiota in this plant defense reaction. We mimicked a caterpillar attack by inflicting mechanical injuries on rice leaves followed by addition of laboratory-reared *Spodoptera frugiperda* caterpillars (6th instar) regurgitation on wounds. First, we observed a significant transcription of some JA pathway markers (JAZ5, Myc2, PR10) upon this treatment. When the same experiment was conducted with caterpillar regurgitation from insects that were fed with antibiotics (ampicillin 100µg/mL, erythromycin 400µg/mL), the transcription of the same markers was twice over-induced. We showed that the antibiotic treatment modified the insect intestinal microbiota. Before treatment, we identified the bacterium *Enterococcus mundtii* and the yeast *Diutina rugosa* by plating on nutrient agar medium of both larvae regurgitation and crushed gut. After antibiotic treatment, the bacterium disappeared. In conclusion, our preliminary results show that the *S. frugiperda* OS induce JA-dependent defense pathways in rice and that bacterial microbiota attenuates this reaction.

Key-words : ‘Plant-Insect Interaction’ ‘*Spodoptera frugiperda’* ‘microbiota’ ‘rice’ ‘Jasmonate’

AgriVectors: a community resource for arthropod vectors and agricultural pathosystems

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ABSTRACT (270/300 words)

Objective: Arthropod vectors of pathogens cause enormous economic losses and are a fundamental challenge for sustainable food production, yet agricultural pathosystems remain an under-served area of research. To develop more effective control of plant pathogens and pest, data pertaining to a disease system needs to be consolidated, made accessible, searchable and amenable to data mining.

Method: The AgriVectors™ platform is an open access and comprehensive resource for growers, researchers and industry who are working on plant pathogens and pathosystems that are spread by arthropod vectors. The portal connects established public repositories with 'pathosystem-specific' data repositories. Current resources include the Asian citrus psyllid, the potato psyllid and the bacterial pathogens they transmit to citrus and Solanaceous plants. Expansion to other important hemipteran vectors (whiteflies, leafhoppers, planthoppers, scale, mealybugs etc.) along with thrips and mites is planned. There is also a capacity to set up private and protected databases for restricted access as needed. The portal is based on the Citrusgreening.org community resource that was developed as a model for systems biology of tritrophic disease complexes. Citrusgreening.org provides resources for the Huanglongbing pathosystem. In addition, it includes a biochemical pathway database for each organism (psyllid/bacterium/citrus). An *Expression Atlas* with proteomics and RNAseq data from psyllids across multiple citrus hosts and a variety of states of infection is also available to identify candidate genes.

Results: To be discussed are plans to expand to diseases of fruit crops (trees /grapevines) in Europe that are transmitted by arthropods, especially phytoplasma and viral pathogens. The AgriVectors portal will extend this model beyond gene-centric omics-data to the broader *Systems Biology Pathosystem-wide* information, with integrated pest management, behavioral, plant health, soil health and climate data to incorporate rapid phenotyping information from research trials, building a foundation for more effective identification and development of solutions to control plant diseases.

Keywords: Arthropods, database, Insect, vector, pathogen, psyllid, open access

Evolved transcriptional responses and their regulation after long-term adaptation of *Bemisia tabaci* to a marginally-suitable host

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Although generalist insect herbivores can migrate and rapidly adapt to a broad range of host plants, they can face significant difficulties when accidentally migrating to novel and marginally-suitable hosts. What happens, at both the genome-wide regulatory and transcriptional levels, if these marginally-suitable hosts must be used for multiple generations before migration to a suitable host can take place, largely remains unknown. In order to study this, we established a multi-generational experimental setup that compared the differences between populations of the whitefly *Bemisia tabaci*, a generalist phloem-feeding species, subjected to cotton (a suitable host) and habanero-pepper (a marginally-suitable host on which the initial survival is 5%). We used reciprocal host tests to find the differences in gene expression and to examine the possible role of DNA methylation in their regulation. Our transcriptomic data revealed that most transcriptional changes in the habanero-pepper adapted population (survival increased to above 60%) were changes in three molecular functions/biological processes: enhanced formation of cuticle structural components, reduced activity of cysteine-type peptidases, mainly cathepsin B proteins that activate plant defenses, and carbohydrate metabolic processes. Our methylation analyses of the DNA of the differentially expressed genes, did not find correlation between the gene's expression levels and direction of change, and their promoters and first exons methylation status. Further studies are required to identify the molecular regulatory mechanism/s that control the distinct transcriptional signature of adaptation to habanero-pepper in *B. tabaci* and test the possible involvement of selection for a subset combination of alleles, complex gene-regulatory networks, molecular chaperon hubs and regulatory microRNAs in the regulation of the process.

Keywords: transcriptome, molecular evolution**,** long-term host adaptation, DNA methylation, *Bemisia tabaci*

Simultaneous feeding by the herbivores *Brevicoryne brassicae* and *Plutella xylostella* alters the transcriptomic profile and induced resistance in *Brassica nigra* plants

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Objective

The defence phenotype plants display after the encounter with herbivore insects is the outcome of a complex reprogramming of their transcriptome. In recent years, the mechanisms that regulate transcriptional responses to herbivore attack have been described for several model plants, but less information is available for ecologically relevant systems with non-model plants attacked by multiple herbivores. Here we present how the simultaneous feeding of specialist herbivores, i.e. the aphid *Brevicoryne brassicae* and caterpillars of *Plutella xylostella*, i) affects the transcriptome of wild *Brassica nigra* (L.) and ii) have consequences for plant resistance to each of the two herbivores.

Method

Black mustard plants were infested with ten adults *B. brassicae* and four *P. xylostella* (L1-L2) in combination or individually. Infested leaves were collected at 3, 6, 24 and 48 hours after the onset of herbivory. A paired-end RNA sequencing was performed using the Illumina NovaSeq 6000 platform. The performance of the two herbivores was measured in the conditions of simultaneous *vs* single feeding after five days from induction.

Results

More than 1600 differentially expressed genes were found to be influenced in the dual feeding compared to un-infested plants, over time. Numerous members of the transcription factor families with a conserved role in regulating responses to herbivores, like WRKY and ERF, were strongly up-regulated in the *B. brassicae*-induced and dual-infested plants. The expression of genes associated to defence pathways regulated by Jasmonic Acid and Salicylic Acid is altered at early stages in the simultaneous infestation with aphids and caterpillars. Performance of *B. brassicae* was reduced when feeding simultaneously with the caterpillars of *P. xylostella*, while *P. xylostella* showed an increased growth in the co-feeding condition with the aphid *B. brassicae*, compared to feeding alone.

Conclusions

In conclusion, these results highlight that simultaneous herbivory of the aphid *B. brassicae* and caterpillars of *P. xylostella* promotes caterpillar performance but reduces aphid performance. The transcriptomic characterisation of these interactions offers an important step forward in describing the mechanisms activated by non-model plants in response to multiple herbivores.

Key words: wild black mustard, plant response, defence pathways, plant-mediated interaction, RNA-sequencing.

Identification of chemoreceptors in *Pieris brassicae* based on genome and transcriptome data

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Objective

Chemoreceptors such as odorant receptors (ORs), ionotropic receptors (IRs) and gustatory receptors (GRs) play critical roles in finding host plants, selecting optimal oviposition sites and avoiding natural enemies in insects. Identifying insect chemoreceptors and understanding their functions will help us reveal the molecular mechanism of perceiving environmental chemical signals in insects and develop more effective pest management and crop protection strategies. Here, we identified chemoreceptors of *Pieris brassicae* based on genome and transcriptome data and speculated function of chemoreceptors according to phylogenetic analysis.

Methods

Chemoreceptors of *Pieris brassicae* were identified by searching the genome and caterpillar transcriptome with chemoreceptors of other lepidopteran species as queries, the redundant results and short sequences were removed in searching results and the left unique sequences were filtered with conserved domain. Most odorant receptor sequences were validated by PCR amplification.

Results

44 candidate ORs, 45 candidate IRs and 17 candidate GRs were identified by searching in *P. brassicae* genome. We then searched caterpillar transcriptome with identified chemoreceptors as queries and filtered results with conserved domain. 20 candidate ORs, 23 candidate IRs and 12 candidate GRs were found in caterpillar transcriptome. Phylogenetic analysis showed that there is a candidate pheromone receptor expressed in caterpillars, 12 antennal IRs were identified including IR8a and IR25a, bitter receptors, sugar receptors and CO_2 receptors were found as well.

Conclusion

We identified chemoreceptors from the genome and caterpillar transcriptome based on bioinformatic methods. The function of some candidate chemoreceptors was predicted by phylogenetic comparison to functional studies in related species. However, the functional characteristics of chemoreceptors still need to be validated by *in vivo* or *in vitro* methods.

Keywords: odorant receptors, ionotropic receptors, gustatory receptors, *Pieris brassicae*

The importance of the glycoside hydrolase 13 genes family for sugar metabolism and host utilization of the phloem-feeding whitefly *Bemisia tabaci*

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Phloem feeders are exposed to sucrose concentrations that are high enough to cause a positive osmotic potential between the ingested sap in the gut and the insect hemolymph. Therefore, the ability to maintain sucrose homeostasis is crucial for phloem-feeders development and survival. In this study, we focused on the polyphagous phloem sap-sucking whitefly *Bemisia tabaci*. Prior genomic and transcriptomic studies in our laboratory revealed that *B. tabaci* has an expanded glycoside hydrolase 13 (GH-13) family containing ~70 members, with few of them being highly expressed. GH-13 enzymes can regulate gut osmolarity through hydrolyzation, isomerization, and transglucosylation of sucrose. In addition, a GH-13 enzyme was previously found to be involved in the ability of *B. tabaci* to detoxify the plant toxic secondary metabolites glucosinolates and cyano-glucosides. To further study the functionality of the GH-13 family in *B. tabaci*, we selected five GH-13 genes with different putative functions based on their expression profiles and the amino acids sequence in their active sites. Each of these genes was silenced separately by feeding adults for five days on artificial diets containing sucrose and double-stranded RNAs (dsRNAs). We examined the silencing effect on the survival and profile of sugars in the honeydew of the silenced insects and the development of their progeny. We found that silencing four of the GH-13 genes caused significant mortality (compared to feeding on dsRNA control) and also affected progeny development. Moreover, metabolomic analyses showed variability in the sugar metabolites pattern among the silenced genes, suggesting that each has a different and specific functionality. Based on these findings, we hypothesize that the GH-13 family in *B. tabaci* holds an important key for understanding the contribution of sugar metabolism to successful host utilization in whiteflies.

Keywords: *Bemisia tabaci*, Glycoside hydrolase 13, Sugar metabolism, Osmoregulation, double-stranded RNA

Drought and endophytes interactively affect herbivore-induced plant volatiles and parasitoid attraction in tomato.

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Fungal endophytes can strongly affect plant-insect interactions. We studied the effect of an unspecialized fungal endophyte, *Acremonium strictum*, on plant direct and indirect defence against a generalist insect herbivore, and how drought modulates this interaction. We hypothesized that the fungus alters the blend of herbivore-induced plant volatiles and thereby the attraction of the herbivore's natural enemies and that these effects are context (drought) dependent.

Roots of young tomato (*Solanum lycopersicum)* plants were inoculated with spores of *A. strictum* or mock-inoculated and grown under well-watered or drought conditions in a greenhouse. Bioassays were performed to test plant direct and indirect defence against caterpillars of the the generalist chewing insect herbivore *Spodoptera exigua*. Plant volatile samples were collected by dynamic headspace adsorptions on Porapak Q. A 6-arm olfactometer was used to test attraction of females of the generalist parasitoids, *Cotesia marginiventris* and *Microplitis rufiventris.* Analysis of plant volatiles was done using GC/Q-TOF.

Our results indicate that herbivory induced a higher production of several plant terpenes, but that inoculation with *A. strictum* partly mitigated the herbivore-induced terpene production, both under well-watered conditions and under drought. The parasitoid *C. marginiventris* tended to be less attracted to *S. exigua*-infested plants when these were inoculated with the endophyte than when they were uninoculated, both under drought and under well-watered conditions. At the same time, *M. rufiventris* was significantly repulsed by infested endophytic plants, especially under drought. More detailed analyses of the full effect of the microbe on plant traits under the different watering regimes are underway.

We conclude that the endophyte negatively interferes with the host searching behaviour of the two parasitoid species, which may be due to its partial repression of herbivore-induced terpenoid plant volatiles. Our results therefore illustrate the important role played by plant-associated microbes in plant-herbivore interactions.

KEYWORDS

Plant-microbe-insect interaction, endophyte, parasitoids, plant volatiles, indirect defence.

Insect-plants interactions in agro-ecosystems and forests in the western Andes of Ecuador

Xavier Chiriboga Morales and Giovanni Onore

Fundación Otonga

ABSTRACT

Ecuador is a tropical country located at the Northwest of South America. Two hotspots of biodiversity spread across Ecuadorian territory (Tumbes-Chocó Magdalena and Tropical Andes) (Rieckmann et al, 2011), making Ecuador a highly diverse country (Miller and Dyer, 2008). Biodiversity in fragile ecosystems such as cloudy forests and paramos are threatened by extractive activities such as oil drilling, mining (Roy et al, 2018) and deforestation (Cuesta et al, 2017). The growth of the agricultural frontier in the western Andes of Ecuador (Stadel, C and Lopez Sandoval, MF, 2005) menaces biodiversity as well. However, biodiversity is key in order to maintain ecosystems balance, provide ecosystem services and secure people's health (Morand and Lajaunie, 2017). On the other hand, agriculture is key to maintain people's livelihoods (Tilman et al, 2011). It is necessary to understand that agriculture needs biodiversity and that the conservation of biodiversity and health (including humans' health) needs a healthy agriculture. Ecuadorian agriculture has relied on the use of synthetic chemicals for the control of insect pests for years. This has lead to the loss of insects that play the role of natural enemies of the insect pests in agricultural landscapes and to the development of resistance to pesticides of the insect pests as well (Decknok et al, 2019). Thus, species and ecological networks disappear even before being discovered. The present project aims to integrate information about tri-trophic interactions between herbivorous insects, their host plants (families Poacea and Fabacea) and their insects' parasitoids in two types of ecosystems: undisturbed forests and agricultural landscapes. GOAL: Here it is presented the project AGROFORES-INSECTACTIONS, we look forward to be contacted by interested partners and potential funding bodies.

Keywords: insect-plants, agro-ecosystems, forests, western Andes, Ecuador

Elucidating the mechanisms of aphid resistance in *Triticum monococcum* L. (Poaceae)

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Previous studies have identified greater aphid resistance in the wheat ancestor *Triticum monococcum* than that observed in modern wheat varieties. The aim of this study is to characterise the antixenotic and antibiotic mechanisms underlying aphid resistance in two *T. monococcum* lines, ie. MDR045 and MDR049, specifically the role of secondary metabolites (SMs). Two hypotheses were tested: (i) induction of aphid resistance in MDR045 and MDR049 is aphid density-dependent and (ii) aphid resistance mechanisms are systematically induced upon aphid feeding in MDR045 and MDR049. The hypotheses were tested using 4-way olfactometry assays to assess grain aphid, *Sitobion avenae*, responses to volatile organic compounds (VOCs) collected by air entrainment, and artificial feeding assays to assess *S. avenae* responses to leaf extracts. VOCs and extracts were collected from plants at 0, 1, 5, 10 and 25 *S. avenae* infestation densities, and *T. aestivum* cv. Solstice and *T. monococcum* MDR037 were used as aphid susceptible controls.

MDR049 and MDR045 VOCs showed aphid density-dependent repellent activity against *S. avenae*. MDR049 had a threshold of 1 aphid, whilst MDR045 had a threshold of 25 aphids, to induce a significant repellent activity. MDR049 and MDR045 leaf extracts were antibiotic against *S. avenae* after 24hrs and 48 hrs by reducing adult survival and number of nymphs. At a 25-aphid infestation, this antibiotic activity was increased locally at the feeding site, reversed at the same leaf as the feeding site and not affected at the different leaf of the feeding site. MDR049 leaf SMs change in trends compared to the other accessions with increasing aphid density and may also react systematically to aphid feeding, confined to the same leaf as the feeding site.

In conclusion, SM changes in MDR049 and MDR045 are aphid-density dependent and may be related to their aphid resistance mechanisms. MDR049 shows signs of a systematic response to aphid feeding, not observed by the other tested accession.

Key words: *Triticum monococcum*, *Sitobion avenae*, secondary metabolites, antixenosis antibiosis.

ABSTRACT TITLE **How gut microbiota may shape sympatric speciation in invasive phytophagous insects**

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ABSTRACT TEXT

Objective

Following the introduction of new host plants, rapid evolutionary changes in invasive phytophagous insects, can sometimes result in sympatric speciation. The underlying processes and facilitation factors are still to be investigated in detail. In this paper, we examined the differences in the gut microbiota of two species of *Costelytra* scarabs (Coleoptera: Scarabeidae: Melolonthinae), one non-invasive (*C. brunneum* (Broun)) and one invasive (*C. giveni* n. sp. Cocca-Abia) for which several ecotypes were analysed.

Method

Third instar larvae were collected from five locations in New Zealand including exotic pastures, native grassland and vineyards. Insects were dissected and the microbiota in their hindgut was analysed using a metabarcoding approach based on the ITS gene region. Microbial community compositions were compared in relation to the scarab species, the location of the sampling and the type of vegetation.

Results

We found significant variations between the invasive and the non-invasive species. Three main groups of bacteria contributed to these differences, namely Oxalobacteracea, Rhizobiles and Porphyromonadacea. Among these bacteria, Porphyromonadacea were systematically present in high proportion in the gut of the invasive species *C. giveni*.

Conclusion

Our results highlighted the presence of bacteria potentially providing advantages in the exploitation of a new host plant in the gut of the invasive species. Such bacteria might have contributed to the ability to feed and benefit from introduced host plants such as clover. This study also revealed significant differences in the gut bacterial communities of four *C. giveni* ecotypes, supporting the hypothesis that part of the gut microbiota in this invasive phytophagous insect is likely to have been acquired horizontally from the newly exploited niche. This preliminary insight into the putative roles of gut bacteria shaping the invasion success of *C. giveni* consolidates previous hypotheses and opens new avenues in term of research toward research towards next-generation biocontrol solutions.

KEYWORDS
Host races, ecotype, sympatric speciation, *Costelytra*, New Zealand

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Conclusion

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KEYWORDS
Host races, ecotype, sympatric speciation, *Costelytra*, New Zealand

Aphid-induced changes in the phloem exudate metabolome of wheat and positive effects on aphid offspring suggest transient niche construction by *Sitobion avenae*

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Abstract

Aphids feed on plant phloem sap, which imposes nutritional challenges on these herbivores. Previous studies revealed that aphids may change the phloem sap metabolome. However, how this affects the offspring of the infesting aphid clone and other clones is less clear. We compared the phloem exudate composition of control and previously aphid-infested plants and assessed the development of aphid offspring, using different aphid clonal lineages of two geographically distinct populations. Wheat (*Triticum aestivum*) leaves were either infested with aphids (*Sitobion avenae*) of clonal lineages belonging to one of two geographically distinct populations or left uninfested. After 10 days, leaf phloem exudates were collected using the EDTA method. Amino acids were measured with HPLC-FLD, while metabolic fingerprinting of putative specialised metabolites was performed using UHPLC-DAD-QTOF-MS/MS. In a separate plant batch, aphids of the aphid clonal lineages that had previously infested the plants were removed and nymphs were redistributed by putting subgroups on control leaves or on leaves previously infected by the same clonal lineage or one of the other population. Aphids were counted for 12 days. Aphid infestation led to changes in the relative proportions of aspartic acid and some metabolic features likely representing specialised metabolites, with some effects being aphid population-specific. During the first four days, nymphs showed higher survival on previously aphid-infested than on control plants. Whether plants had previously been infested by aphids of the same clonal lineage or a lineage of the other population had no effect. At later time points, the aphid colony sizes did not differ between treatment groups. The aphid-induced metabolic changes in wheat phloem exudates and the initially higher nymph survival on previously aphid-infested plants may indicate a transient niche construction by the aphids. However, niche choice, which may also contribute to aphid success, was largely excluded by confining aphids to single leaves.

Keywords

Aphids, phloem, amino acids, specialised metabolites, niche construction

Insect frass – effects on plant growth and herbivore performance

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Abstract

Background/objective: Insects are currently produced for their use as a protein source in food and feed and frass is the main by-product. Frass consists of insect faeces mixed with moulting skins. As insect production grows, the amount of frass will grow as well. In the context of a circular economy and the concept of “zero waste“, it is necessary to valorise all components of production, including frass. This study investigates the effect of frass as soil amendment on plant growth and resistance to insect herbivory.

Method: In two greenhouse experiments, soil was amended either with frass of black soldier fly (BSF) (*Hermetia illucens* L.) larvae, frass of mealworm (*Tenebrio molitor* L.) (2g kg^{-1}), or unamended (control). Plant growth over time, herbivory and development of diamondback moth (DBM) *Plutella xylostella* larvae were measured on *Brassica rapa* plants.

Results: Our results show that number of leaves and leaf area differed significantly ($P<0.05$) among treatments and time points. At 21 days, plant growth differed and the difference had increased at 42 days after germination. Compared to the control, frass of BSF larvae consistently suppressed plant growth. Mealworm frass-treated plants showed significantly ($P<0.05$) higher growth than the BSF frass-treated plants, and were similar to plants growing in unamended soil. In the first trial, pupation of DBM larvae was higher ($P<0.05$) on mealworm frass-treated plants than in the control plants and the BSF frass-treated plants had the lowest ($P<0.05$) pupation of DBM larvae. Leaf feeding damage on *B. rapa* plants was generally high (average damage score of 5 on a scale of 7) in all treatments.

Conclusion: Taken together, our results show that frass of different insect species added to the soil impacts plant growth and insect performance differently. The implication of these results for processing of frass prior to amendment to avoid plant growth inhibition will be discussed.

Keywords: insect frass, plant fertilizer, plant growth, plant resistance, insect herbivores

A root-feeding herbivore suppresses indirect plant defences to avoid elevated predation risk.

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It has been well documented that herbivore feeding damage-triggers the production of herbivore-induced plant volatiles (HIPVs), which can protect plants directly by repelling herbivores or indirectly by recruiting natural enemies that kill herbivores. However, our understanding of root HIPVs and their ecological functions in soil environments remains limited. We investigated the role of HIPVs from roots of cucumber plants (*Cucumis sativus*) damaged by cucumber beetle larvae (*Acalymma vittatum*) in mediating foraging decisions of entomopathogenic nematodes (*Heterorhabditis bacteriophora*; EPNs). We also quantified how these volatiles and corresponding EPN recruitment change over the course of herbivory.

We characterized volatile compounds produced by larvae-damaged roots, mechanically damaged roots, and undamaged control roots, following 24 hours and 7 days of continuous wounding. Volatiles were collected using dynamic headspace sampling and analysed with GC-MS. We also quantified EPN preference for volatiles from herbivore-damaged or control plants using 2-choice belowground olfactometers.

We found that both cucumber beetle herbivory and mechanical wounding induced greater production of volatiles from *C. sativus* roots after 24 hr, compared to undamaged control plants. However, after 7 days of continuous damage, *A. vittatum* herbivory suppressed root volatile production, while mechanical wounding continued to induce higher volatile production. Furthermore, we found that EPNs were initially attracted to cucumber root HIPVs after 24 hr of damage, but after 7 days of herbivory, EPNs did not differentiate between herbivore-damaged and control plants.

Taken together our results indicate that HIPVs from *C. sativus* roots serve as indirect defences against *A. vittatum* larvae through the recruitment of EPNs. However, *A. vittatum* larvae can overcome these defences by suppressing HIPVs.

Keywords: Belowground chemical ecology, indirect plant defences, multitrophic interactions, root volatiles, herbivore-induced plant volatiles

Does Autophagy Contribute to Plant's Defense Against Insects?

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Plant autophagy, the vacuolar degradation of cytoplasmic constituents, was shown to function at basal level under normal growth conditions, and to be induced under abiotic and biotic stresses. While autophagy was shown to be involved in plant's response to fungi, bacteria, and viruses, its role in insect's infestation is unknown. To understand the relation between herbivores and the autophagy machinery, we focused on two model organisms, *Arabidopsis thaliana* plants and *Myzus persicae* (green peach aphid). We compared changes in gene expression, protein levels, metabolic profiles in autophagy-defective (*atg5.1 and atg7.2*) and *ATG8E* overexpressing *Arabidopsis* mutants relative to wildtype following 6 hours of aphid feeding during day or night. We also measured aphid's bodyweight and fecundity. Higher ATG8E protein levels and ATG gene expression were observed upon aphid's infestation of plants in either day or night. Aphid's body weight was lower in ATG mutants relative to WT and *ATG8E* overexpressing plants. In accordance with bodyweight, the aphid's reproduction was lower in ATG mutants compared to WT and *ATG8E* overexpressing plants. These results suggest that biotic stress such as aphid feeding induces autophagy in plants. Nevertheless, whether insect-induced autophagy contributes to the plant's defense directly or indirectly will be further studied. The metabolic results will be available at the time of the conference.

Keywords: Plant Autophagy, plant-insect interaction, *Arabidopsis thaliana*, *Myzus persicae*.

Insect-Plant interactions in the future: elevated atmospheric carbon dioxide suppresses silicon defences in wheat, but herbivore resistance persists

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Objective

As grasses, cereals use the uptake and deposition of silicon (Si) in tissues as a mechanism to resist insect herbivore attack. Previous research suggests that predicted increases in atmospheric carbon dioxide (CO_2) cause some grasses to accumulate less Si. Our objective was to understand whether elevated levels of atmospheric CO_2 (e[CO_2]) suppressed Si defences in wheat, in favour of C-based defences (phenolics), and whether this impacted an insect herbivore.

Method

We quantified how ambient and e[CO_2] affected plant growth and leaf concentrations of carbon (C), nitrogen (N), Si and phenolics in three cultivars of wheat (*Triticum aestivum*) receiving three levels of Si supply (0, 1 or 2mM). We tested how these changes affected relative growth rates (RGR) and mouthpart (mandibular) wear of the global pest *Helicoverpa armigera*.

Results

Averaged across cultivars, e[CO_2] promoted plant growth by 33% and caused plants to switch from Si (–19%) to phenolic (+44%) defences. e[CO_2] increased leaf C concentrations, which were positively correlated with phenolics and negatively correlated with leaf Si concentrations. Despite the lower levels of Si under e[CO_2], *H. armigera* RGR was reduced by at least 33% on plants supplied with Si, irrespective of CO_2 levels. Mandible wear was c. 30% higher when feeding on Si supplemented plants compared to those feeding on plants with no Si supply.

Conclusion

We conclude that higher C availability under e[CO_2] reduces silicification and causes wheat to increase concentrations of phenolics. Si supply, even at the lower level, suppressed the growth of *H. armigera* under both CO_2 regimes, suggesting that increasing Si levels in wheat may be beneficial for pest management.

Keywords: climate change, crop pests, herbivory, plant defence, silica

Double Trouble:
Effects of water stress on plant responses to insect herbivores

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Objective

Plants have to cope with multiple types of stress simultaneously. The responses to these stressors can interfere with each other, amplifying their negative effects. In this study we focus on the effects of water stress (drought and waterlogging) on direct and indirect resistance of plants against insect herbivory. Furthermore, every plant species is differently adapted to stressors depending on its ecological niche. Therefore, we compared three closely related *Rorippa* species that differ in adaptations to dry or waterlogged habitats.

Method

We measured the performance of four insect herbivores (the aphids: *Myzus persicae, Lipaphis erysimi* and caterpillars of *Plutella xylostella, Pieris brassicae*) on three different plant species under three different water conditions (drought, well-watered, waterlogged) as proxy for plant direct resistance. To study indirect resistance, we performed a two-choice assay with the parasitoids *Aphidius ervi* and *Diadegma semiclausum* parasitizing *Myzus persicae* and *Plutella xylostella* respectively. Parasitoids choose between plants that were infested by host herbivores and received one of the three different water conditions.

Results

Aphids performed better on drought stressed plants on two of the three plant species while on the third aphids performed equally well under all water conditions. Caterpillar performance was not affected by the different water conditions regardless of plant species. Drought had an adverse effect on the attraction of *A. ervi* parasitoids for one of the three plant species. In other cases, including all experiments with *D. semiclausum*, parasitoids were attracted to stressed plants regardless of whether this stress originated from suboptimal amounts of water or insect herbivory.

Conclusion

These studies show that water stress can disrupt both direct and indirect resistance against insect herbivores of the plant. The exact consequence of water stress on responses to insect herbivores, however, depend on the plant species' adaptations to water stress.

Keywords: water stress, simultaneous stress, direct resistance, indirect resistance,

ABSTRACT TITLE

Differences in the osmoregulation strategies of two *Bemisia tabaci* (Hemiptera: Aleyrodidae) species – insights from glycomic analysis

AFFILIATIONS

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ABSTRACT TEXT

Multiple studies tried explaining the host range differences among species within the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae), but none of them addressed the insect's osmoregulation strategy, despite its cruciality for successful phloem-feeding (continuous exposure to excess sugar). To regulate gut osmotic potential, *B. tabaci* stabilizes and ultimately excretes the excess sugar as sucrose isomers or oligosaccharides. Using LCMS, we applied here a comparative metabolomic approach, to profile the honeydew excretions of two *B. tabaci* species feeding on different plants and artificial diets: Sub-Saharan Africa 1 (SSA1) that infests cassava plants, and the generalist species MEAM1 that cannot colonize cassava successfully. Ultimately, we asked whether the two species differ in their osmoregulation strategy and whether this may be associated with their plant host utilization differences. Individuals from the two species were transferred from a common host (aubergine) to either cassava or broccoli (more suitable for MEAM1), or to a range of sucrose artificial diets (0.15M-2M). Sucrose isomerization products were more variable in SSA1 than in MEAM1 in most conditions. In addition, SSA1's transglucosylation products on each plant were longer than MEAM1's, and SSA1's transglucosylation response to sucrose in artificial diets was stronger than MEAM1's. Interestingly, MEAM1's response to the transition to cassava showed a strong decrease in the primary sucrose isomerization reaction, that did not seem adaptive, while SSA1 exhibited a more sophisticated response, with many relative changes in honeydew components. Finally, trehalose traces in SSA1's honeydew were consistently higher than those in MEAM1. Taken together, our data imply that the osmoregulation strategy of SSA1 is affected by the plant/diet more than that of MEAM1. In addition, the higher levels of trehalose in the SSA1 honeydew may suggest higher hemolymph trehalose concentrations that may protect it from various types of stress, potentially helping SSA1 insects to survive on cassava plants.

KEYWORDS

Bemisia tabaci, Osmoregulation, Isomerization, Transglucosylation, Cassava

ABSTRACT TITLE

A benzoxazinoid-derived methoxy-nitrophenol deters western corn rootworm larvae

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ABSTRACT

Plants produce a staggering variety of metabolites, which have diverse and often intertwined functions in growth, development and interactions with other organisms. Many plant metabolites and/or their biological functions, however, remain to be characterized. Like most grasses, maize (*Zea mays*) produces benzoxazinoids, which are nitrogen-containing, aromatic compounds with broad antixenotic and antibiotic properties that serve to protect plants from herbivores and pathogens. In addition, one of the main benzoxazinoids, DIMBOA, is secreted from roots to sequester and retrieve iron from the soil, thus fulfilling an essential role in maize growth. Here we report on the discovery and biological relevance of 5-Methoxy-2-Nitrophenol (5M2NP), which represents a class of metabolites not previously known from plants. Given the structural similarities between benzoxazinoids and 5M2NP, we hypothesized a shared biosynthetic origin for these metabolites. Accordingly, using SPME-GC-MS analysis of ground tissues, we consistently detected 5M2NP in roots and leaves of wild type maize, yet only trace amounts in *bx1* mutants, which are defective in the production of benzoxazinoids. Moreover, we managed to restore 5M2NP production in *bx1* by supplementing ground roots with DIMBOA. Next, we sought to determine the biological function of 5M2NP. Since most benzoxazinoids are defensive metabolites and 5M2NP is volatile, we hypothesized it may deter herbivores. Indeed, experiments with belowground olfactometers demonstrated that western corn rootworms (*Diabrotica virgifera virgifera*), a major insect pest of cultivated maize, clearly preferred odours from roots of control plants over those of roots from plants supplemented with 5M2NP. We found a similar preference pattern using choice assays in petri dishes with detached roots, supplemented or not with 5M2NP. Taken together, we have identified a novel plant metabolite in maize, 5M2NP, which is derived from DIMBOA and deters an agriculturally important insect pest.

KEY WORDS

maize, methoxy-nitrophenol, benzoxazinoids, western corn rootworm, antixenosis

MORPHOLOGICAL AND CHEMICAL FACTORS RELATED TO WESTERN FLOWER THRIPS RESISTANCE IN THE ORNAMENTAL GLADIOLUS

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ABSTRACT TEXT

Objective

Understanding the mechanisms involved in host plant resistance opens the way for improved resistance breeding programs by using the traits involved as markers. Pest management is a major problem in cultivation of ornamentals. Gladiolus (*Gladiolus hybridus* L.) is an economically important ornamental in the Netherlands. Gladiolus is especially sensitive to attack by western flower thrips [*Frankliniella occidentalis* (Pergande) (Thysanoptera:Thripidae)]. The objective of this study was, therefore, to investigate morphological and chemical markers for resistance breeding to western flower thrips in Gladiolus varieties.

Method

We measured thrips damage of fourteen Gladiolus varieties in a whole plant thrips bioassay and related this to morphological traits with focus on papillae density. Moreover, we studied chemical host plant resistance to using an eco-metabolomic approach comparing the ^{1}H NMR profiles of thrips resistant and susceptible varieties representing a broad range of papillae densities.

Results

Thrips damage varied strongly among varieties: the most susceptible variety showed 130 times more damage than the most resistant one. Varieties with low thrips damage had shorter mesophyll and epidermal cells, as well as a higher density of epicuticular papillae. All three traits related to thrips damage were highly correlated with each other. We observed a number of metabolites related to thrips resistance against thrips: two unidentified triterpenoid saponins and the amino acids alanine and threonine. All these compounds were highly correlated amongst each other as well as d to the density of papillae.

Conclusion

These correlations suggest that papillae are involved in resistance to thrips by producing and/or storing the compounds causing thrips resistance. Although it is not possible to distinguish the individual effects of morphological and chemical traits statistically our results show that papillae density is an easy marker in Gladiolus breeding programs targeted at increased resistance to thrips.

KEYWORDS
Gladiolus; *Frankliniella occidentalis*; host plant resistance; morphological markers; mesophyll; epidermis; papillae; eco-metabolomics

Elevational gradients in constitutive and induced oak defences based on individual traits and their correlated expression patterns

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Elevational gradients are useful tools for disentangling the biotic and abiotic drivers of plant–insect interactions. Traditional theory holds that greater herbivory pressure under warmer and less variable climatic conditions characteristic of low elevations should select for greater investment in plant defences. However, the generality of this prediction has been called into question by recent studies reporting contrasting patterns of variation in herbivory and plant defences with elevation. A source of inconsistency might be that most studies have looked at individual plant traits instead of assessing multiple defensive traits and their correlated expression patterns. In addition, the role of abiotic factors associated with such patterns has been barely explored. To address this knowledge gap, we studied elevational clines in direct (phenolic compounds) and indirect (volatile organic compounds) constitutive defences and their inducibility after feeding by a specialist beetle *Altica quercetorum* in 18 populations of *Quercus pyrenaica*. We tested for: 1) elevational clines in constitutive direct and indirect defences, 2) patterns of correlated expression between defences and 3) associations between any such clines and climatic factors. We observed a decrease in constitutive direct defences (lignins and hydrolysable tannins) with increasing elevation. In contrast, we found no elevational gradient for constitutive indirect defences (volatile organic compounds) or the inducibility of direct or indirect defences when looking at groups of compounds. However, we found an increased induction of two monoterpenes (α-fenchene and camphene) at higher elevation. Furthermore, we show a significant pattern of co-expression of constitutive and induced phenolics across populations, which weakened with increasing elevation. Finally, we found no association between climatic factors and trait expression patterns across elevations. Overall, these findings show that assessing elevational shifts in trait correlated expression patterns and their underlying mechanisms can increase our understanding of plant defence evolution and plant–herbivore interactions along environmental gradients.

Keywords: geographic gradients, insect herbivory, plant defence syndromes, phenolic compounds, volatile organic compounds

BUTTERFLY VISITORS, FLORAL ATTRIBUTES AND STANDING NECTAR CROP FROM TROPICAL PLANTS OF INDIA

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Abstract

Systematic studies focussing on the role of butterflies in pollination are scarce, though they are considered next to honey bees as important pollinators. Data on butterflies and associated floral attributes such as flower morphology, flower colour, type, odour, primary attractants in the flower and sexual organs of twenty plant species were collected through regular visits from urban and rural landscapes around Pune city, Maharashtra, India. Additionally, to ascertain the role of butterflies in pollination, the data on pollens that are attached to the proboscis and other body parts were also collected by conducting visits to areas of Western Ghats, a global biodiversity hot spot. Pollen shape, pollen unit, aperture type and exine ornamentation were studied using Scanning Electron Microscopy. In total, forty four butterfly species were observed foraging on different plant species. Nymphalidae family with nineteen species was the most common followed by members of pieridae (11), lycaenidae (6), papilionidae (6) and hesperiidae (2). Three butterfly species were observed visiting more than ten plant species, with *Papillio demoleus* (Common Lime butterfly) recorded on thirteen plant species, the highest amongst all butterfly species. Highest number of visitors i.e., thirty eight different butterfly species were observed foraging on *Lantana camara* with multi coloured flowers. The range of average nectar quantity per flower varied from 0.06 µL to 9.3 µL It was observed that butterflies carry pollens of forty four different plant species. Spheroidal pollens was the most common shape (54%) and monad the most commonly observed pollen unit (97%). 40% species possess tricolpate pollens whereas echinate exine pattern was recorded in 27% species. Present study highlights the role of butterflies as significant contributors to the pollination. More efforts are required to study other factors such as nectar composition, colour preference and floral rewards that may influence butterfly flower choice.

Key words: Butterfly diversity, flower visitors, floral morphology, pollen diversity, standing nectar crop

Is defence of *Echinothrips americanus*-larvae the key to lack of their biocontrol?

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The aim of our research is to investigate the possibility of biological control of the plant pest *Echinothrips americanus* and discern why biocontrol of this thrips is so challenging. Several authors reported natural enemies that showed potential for control of *E. americanus* under laboratory conditions, but there has been no success with controlling them in greenhouses. We focused on the natural enemy *Cosmolaelaps* sp., a predatory mite that was found in the litter layer in a rose greenhouse in the Netherlands. In laboratory experiments, *Cosmolaelaps* preyed on larvae and adult *Echinothrips*. We therefore tested the ability of *Cosmolaelaps* to prey and control thrips populations on intact bean and sweet pepper plants. In all these experiments, no effect of the predators on thrips densities was found, although we did observe *Cosmolaelaps* on the plants. We speculated that *E. americanus* may show some antipredator behavior on plants, thus preventing or reducing the ability of *Cosmolaelaps* to prey on it. We conducted some preliminary experiments on bean leaf disks and found that both larvae and adult thrips did counterattacked adult *Cosmolaelaps*. The counterattack behavior consists of the thrips larvae hitting the predators with their abdomen, a behavior that has been described for other thrips species. We observed that predators seemed to avoid further attacks of the thrips after such counterattacks. We did not see such decrease in predation in our previous laboratory experiments, possibly because of the lack of alternative food sources in the lab experiments, and their presence on the plants or in the soil. In conclusion we suggest that the key for effective biocontrol of *Echinothrips* is to find predators that can prevent or are resistant to this antipredator behaviour.

Key words: biocontrol, *Echinothrips americanus*, *Cosmolaelaps* sp., antipredator behavior.

Indirect plant-mediated interactions among parasitoids

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Objective
We investigated whether sequential or simultaneous feeding by two species of herbivores hosting a parasitoid results in plant-mediated interactions among parasitoids. We address whether parasitoids manipulate plants for their own benefit.

Method
In the first experiment, we used two host/parasitoid systems (*Mamestra brassicae/Microplitis mediator* and *Pieris rapae/Cotesia rubecula*) sequentially or simultaneously feeding on *Brassica oleracea* plants. Then, we measured the development time, weight and mortality of the two parasitoids after developing on plants induced by healthy or parasitized herbivores from the two systems relative to performance on undamaged plants.
In the second experiment, *Pieris brassicae* caterpillars parasitized by *Hyposoter ebeninus* or *Cotesia glomerata* were fed with leaf pieces of *B. oleracea* during their entire lifespan. Leaves were cut from plants that were either not induced or continuously induced by healthy or parasitized caterpillars (by *H. ebeninus*, *C. glomerata* or *C. rubecula*). We measured the development time, weight and mortality of parasitoids that emerged from caterpillars.

Results
First, we found an asymmetrical plant-mediated effect among parasitoids from different systems that fed sequentially on plants, but no effects were found when they were feeding simultaneously. Second, the two parasitoid species did not perform better when their host was fed with leaves from plants continuously induced by a conspecific parasitoid. However, parasitoid species asymmetrically affected each other's performance through plant-mediated interactions.

Conclusion
We show that indirect plant-mediated interactions among parasitoids sequentially feeding on the same plant can also happen between two different host/parasitoid systems, suggesting that this type of interaction is widespread. However, if the two parasitized herbivores feed simultaneously on the same plant, no plant-mediated effect is observed. Finally, the results from our second experiment do not support the hypothesis of parasitoids manipulating plant responses for their own benefit, suggesting that indirect plant induction by parasitoids may be a by-product of host manipulation.

KEYWORDS
Tritrophic interactions, induced plant response, parasitoid performance, plant manipulation.

Pollinator behaviour changes after encountering diseased plants during foraging bouts

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Objective: Pollinator foraging behaviour plays a crucial role in transmission of pollinator-borne plant diseases. However, very little is known about the way pollinators respond to encountering diseased (often less rewarding) plants during their foraging bouts. We investigated whether pollinators adjust visitation frequencies, lengths of visits, and flight distances in diseased plant patches.

Method: We observed behaviour of pollinators foraging in experimental patches of pre-grown plants of *Dianthus carthusianorum* that were either healthy or infected with anther smut disease *(Microbotryum violaceum*). We also measured floral rewards and visual attractants to identify the differences between healthy and diseased plants.

Results: Pollinators showed only weak initial avoidance of diseased plants, but subsequently probed fewer flowers on the diseased plants than on the healthy ones. Additionally, we found striking differences in foraging behaviour of main functional pollinator groups. The pollen-foraging pollinators (solitary bees and hoverflies) displayed stronger response to diseased plants than the nectar-feeding butterflies did, presumably because pollen was absent in diseased plants, while nectar was still present although in reduced amounts.

Conclusion: Since diseased flowers offered lower rewards, the observed pollinator behaviour was consistent with the prediction of optimal foraging models that pollinators should spend less time exploring less rewarding patches or plants.

Keywords: optimal foraging, pollinator-transmitted pathogen, anther smut, pollinator preferences

ABSTRACT TITLE

The mechanisms by which nitrogen addition affects grassland insect communities and their impact

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ABSTRACT TEXT

Objective

Understanding the mechanisms by which global change affects insect consumer communities is critical to offset their declines. Nitrogen is a key global change driver that affects insect consumers through changes in plant defences and nutrient levels. The relative importance of these different mechanisms in driving consumer abundance, diversity and impact at a community scale is poorly understood and is likely to vary depending on insect feeding guild.

Method

To address these questions, we assess insect communities in a large grassland experiment (PaNDiv), which factorially manipulates nitrogen, plant diversity, plant functional composition and foliar pathogen exclusion. Within these 216 unique communities we assess leaf physical and chemical defence traits, nutrient levels and traits related to the leaf economics spectrum, and link shift in leaf traits to insect herbivore abundance/diversity measures and mouthpart traits. These community level interactions are all brought together using structural equation modelling, where we assess the direct and indirect effects of nitrogen enrichment on traits and overall herbivore impact on ecosystem functioning (productivity).

Results

First results from this analysis suggest that nutrient ratios play an important role in determining the impact which insect consumers have on grassland plant productivity. Additionally, the shift in plant functional composition appears to be the most inflectional driver of community leaf traits when compared to the other direct and indirect affects of nitrogen addition.

Conclusion

Initial results suggest stoichiometry and plant functional composition both play an important role in determining interactions between plants and insect herbivores and their subsequent impact. However, this study is limited in time and space to one study location and only two time points within the same year. Yet this study’s contribution to disentangling the mechanisms by which nitrogen affects grassland insect communities and their impact, is nonetheless beneficial to furthering our understanding of global change impacts.

KEYWORDS

community ecology, trait matching, global change biology, ecosystem functioning, grasslands

Microbe-Induced Resistance against the tomato leaf miner *Tuta absoluta*: Scaling up from lab to field applications.

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Objective

In the present study we investigated the ability of different soil-borne microorganisms including bacteria and fungi, to induce systemic resistance against the tomato leaf miner *Tuta absoluta* under different settings, and explored the possible underlying mechanisms.

Method

Tomato (*Solanum lycopersicum*) plants were inoculated with five different beneficial microorganisms: *Bacillus amyloliquefaciens*, *Pseudomonas azotoformans*, *Trichoderma harzianum*, and the arbuscular mycorrhizal fungi *Rhizophagus irregularis* and *Funneliformis mosseae*. The impact of microbial inoculations on *T. absoluta* development was evaluated by quantifying the proportion of larvae reaching adult stage under controlled conditions or evaluating the natural incidence under commercial production conditions. To explore the underlying mechanisms, we performed an untargeted metabolomic analysis using ultra-performance liquid chromatography tandem mass spectrometry (UPLS-MS) on leaf samples.

Results

Under controlled conditions, the proportion of *T. absoluta* reaching adults was lower when feeding on plants inoculated with *B. amyloliquefaciens*, *T. harzianum*, *R. irregularis* and *F. mosseae*, evidencing microbe-induced resistance triggered by these microorganisms against the leaf miner. Under commercial production conditions, the three fungi tested, *T. harzianum*, *R. irregularis* and *F. mosseae,* reduced the proportion of plants with mines, supporting consistency from the lab to field applications. An untargeted metabolomic analysis revealed that the three fungal treatments had an important impact on the plant metabolome and modulated the plant response to *T. absoluta*.

Conclusion

Our study provides insights on microbe-induced resistance against *T. absoluta,* likely associated to the impact of microbial colonization on plant metabolic reprograming in

response to *T. absoluta*. *T. harzianum*, *R. irregularis* and *F. mosseae* showed consistent effects in the different experimental set ups, ranging from lab to field, revealing their great potential for the biocontrol of this major pest.

Keywords: Metabolomics, microbe-induced resistance, *Tuta absoluta*

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Lessons from silicon-enhanced root nodulation in a symbiotic legume: Concepts, causes and consequences on an insect herbivore

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Plant silicon (Si)-based defence is potent against insect attacks. However, studies in plant Si majorly focusses on grasses (Poaceae) and neglects the other important plant groups, such as legumes (Leguminosae/Fabaceae). Legumes have evolved a symbiotic association with nitrogen-fixing bacteria (rhizobia), resided in root nodules. Our study aims to examine the impacts of Si enrichment on a symbiotic legume (*Medicago truncatula*) with rhizobia (*Ensifer meliloti*) and their subsequent consequence on a foliar-chewing insect (*Helicoverpa armigera*). We used *M. truncatula*-*E. meliloti* pairs as a model system in which the rhizobial partners differed in the symbiotic efficacy on the host plant, namely Sm1021 ('low-efficiency') and Sm1022 ('high-efficiency') strains, enriched by Si (+Si) or untreated (-Si) under N-limitation in a controlled glasshouse experiment. Moreover, to better understand the underlying mechanism of how Si affected the legume-rhizobia symbiosis, we employed some chemical/analytical tools to quantify chemical properties within root nodules (flavonoids) and foliar tissues (free amino acids, soluble protein, elemental C, N and Si). Finally, we tested whether Si effects on plants associated with rhizobia impacted the insect relative growth rate as a proxy for plant resistance. Generally, Si enrichment caused significant promotions in, for example root nodulation (i.e. nodule abundance and flavonoids) and foliar nitrogenous contents (i.e. amino acids, soluble protein and total N) but not in foliar Si. We also found that rhizobial efficacy (low vs high) mattered and influenced the magnitude of Si effects on multiple traits. However, Si-enhanced primary chemistry, for example total foliar amino acids did not reduce plant resistance against *H. armigera*. Collectively, our study indicates that Si enrichment generates positive effects on *M. truncatula*, particularly whose association with rhizobia is relatively poor without compromising plant resistance to a polyphagous insect *H. armigera*.

KEYWORDS: Bacteria, insect, legume, root nodulation, silicon

Phytoplasma infection reduces cranberry resistance against aboveground and belowground insect herbivores

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Objective
Phytoplasma infection can affect not only a plant's interactions with aboveground herbivores but also its interactions with belowground herbivores; however, these latter interactions have rarely been studied. In cranberry (*Vaccinium macrocarpon*) vines, phytoplasma infection causes false blossom disease. Here, we investigated the effects of phytoplasma infection of cranberries on the performance of the foliar feeder gypsy moth (*Lymatria dispar*) and the root feeder oriental beetle (*Anomala orientalis*).

Method
We measured the growth, survival, and consumption by larvae of *L. dispar* and *A. orientalis* on phytoplasma-infected and uninfected cranberries. We tested these effects on two cranberry varieties (Ben Lear and Crimson Queen). We also assessed the effects of phytoplasma infection on shoot and root growth and nitrogen content.

Results
In general, larvae of the two herbivore species grew bigger and consumed more plant tissue of phytoplasma-infected compared to uninfected plants, regardless of the cranberry variety. Phytoplasma infection reduced the growth of cranberry shoots and roots, and nitrogen concentrations in both shoots and roots of infected plants were higher than those of uninfected plants.

Conclusion
Together, our findings suggest that phytoplasma infection enhances the nutritional quality in shoots and roots of cranberries, which may explain the observed enhancement of aboveground and belowground herbivore performance on infected plants. These findings provide new insights into the interactions among plants, phytoplasma infection, and aboveground and belowground insect herbivores.

KEYWORDS
Gypsy moth larvae, soil grubs, performance, consumption, nitrogen content

Unravelling the impact of herbivore attack on plant reproduction: Direct and indirect pollination-mediated effects of herbivory on plant male and female fitness.

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Reproduction in flowering plants is generally limited by two factors: pollination and resource availability. Pollen- and resource limitations can be caused by herbivore attack. Herbivore attack damages photosynthetic tissues and induces the production of costly defences. Herbivore-induced plant responses may reduce the attractiveness of flowers and the attraction of pollinators. It is currently unclear if plant male and female fitness are affected to the same extent by direct and indirect effects of herbivory via altered pollination.

In this study, we investigated direct and indirect pollination-mediated effects of herbivore attack on the male and female fitness of *Brassica nigra*. In a common garden experiment, we infested plants with caterpillars of *Pieris brassicae*. For both infested and uninfested plants, we hand pollinated one branch with flowers, while another was marked for open pollinated by naturally occurring pollinators. We hand pollinated individual flowers of uninfested plants with pollen from infested and uninfested plants. In addition, we assessed effects of three different herbivores on pollen germination rates in the laboratory.

We found that indirect rather than direct effects of herbivory reduce plant female fitness: open-pollinated branches of herbivore-infested plants produced less seeds than uninfested control plants, or hand-pollinated branches of infested and uninfested plants. Interestingly, these effects varied over the season. Herbivory also reduced plant male fitness: hand-pollinated flowers of uninfested plants with pollen from infested plants produced less seeds compared to flowers pollinated with pollen from uninfested plants. In addition, damage by two of three different herbivores reduced pollen germination in the laboratory.

Our study shows that herbivory can directly and indirectly via altered pollination reduce plant male and female fitness. Characterization of direct and indirect effects of herbivory on plant male and female fitness aid to understand plant evolution in complex multispecies communities including antagonistic and mutualistic community members.

ABSTRACT TITLE

Understanding the role of insect herbivory on the balance between cross- and self-pollination in *Brassica*

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ABSTRACT TEXT

Objective

Insect herbivores can cause great damage to plants in the field, with consequences for plant reproduction and seed yield. Apart from direct damage to flowers and seeds, herbivores affect plant reproduction indirectly by changing plant-pollinator interactions or by reducing outcrossing rates due to favouring self-pollination under stressful conditions. The aim of my PhD is to understand how herbivory affects outcrossing through its effect on pollinators and self-incompatibility mechanisms in *Brassica rapa*. This project contributes to the emerging research field of plant-mediated interactions between herbivores and pollinators to elucidate the connection between plant defence and reproductive strategies.

Method

I take a multidisciplinary approach of field ecology, greenhouse experiments and molecular studies. *Brassica rapa* is used as the model system, as lines with varying levels of self-incompatibility (SI) are available. The herbivores used are all pest species commonly found on *Brassica:* aphids (*Myzus persicae, Brevicoryne brassicae)* and caterpillars (*Plutella xylostella, Mamestra brassicae).*

I use field studies to determine the effect of herbivory on the composition of the pollinator community, pollinator visitation rate, and pollinator behaviour. The amount in which a pollinator species contributes to the cross-pollination opportunities for the plant depends on all of these factors. To answer these questions requires both extensive pollinator studies and genetic analysis of offspring to determine the outcrossing rate.

In the greenhouse and lab I take a closer look at the effect of herbivory on SI. I will uncover if and to which extend herbivory has an effect on the level of SI in *Brassica*. I will use greenhouse studies to understand the size of this effect, as well as microscopy, qPCR and RNAseq to understand the underlying mechanisms of herbivore-induced reduction of SI.

KEYWORDS
Plant reproduction, herbivore-induced plant responses, insect community, pollinators, self-incompatibility

Title: Fungicide effects on strawberry floral scent and pollination

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Strawberry (*Fragaria x ananassa* Duchesne, Rosaceae) is an important crop and insect pollination markedly increases the fruit yield. Yet, strawberries are also one of the most vulnerable fruits, and numerous agrochemicals are approved for cultivation. However, many pesticides cause measurable changes to the plant metabolism, which may indirectly affect plant-pollinator interactions, for example, by impeding the ability of pollinators to locate the flowers. Moreover, pesticides such as fungicides may alter the diversity and abundance of the microbial community, which significantly contributes to the floral scent. We investigated the effects of two commonly used systemic fungicides, a combi-preparation of a pyrimidine and a phenylpyrrole and one that contains copper as active substance, on the interaction between strawberry and pollinators. We analysed the composition of headspace volatile organic compounds (VOC) of flowers of differently treated plants and measured the attractiveness of flowers to bumblebees (*Bombus terrestris*) with and without fungicide treatment. We found that fungicide treatments alter the flower VOC composition and the attractiveness of flowers to bumblebees. The altered VOC composition could be caused by metabolic changes in the plant as well as compositional changes in the floral microbial community. Overall, our study highlights a so far neglected effect of fungicides on pollinator-dependent crops, namely the alteration of floral headspace VOC and the resulting attractiveness of strawberry flowers to bumblebees, which likely affects the crop yield.

KEYWORDS: fungicide, TD-GC-MS, microbiome, behaviour, pollination

Deadly attractive: The potential of endophytic entomopathogenic fungi in dead-end trap cropping of aphids in vegetable crops

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Objective

An increasing number of studies has shown that entomopathogenic fungi not only act as insect pathogens, but can also endophytically colonize the tissues of certain plants. As such they can promote plant growth, stimulate plant health, and increase resistance against both abiotic and biotic stresses, including insect pests. However, so far most reports on the effects of endophytic entomopathogenic fungi on insect herbivores have concentrated on grasses, while only a few studies focussed on economically important crops. The objective of this study was to investigate whether and to which extent endophytic entomopathogenic fungi can be used to reduce the population of economically important pest insects on vegetable crops.

Method

Specifically, we tested effects of inoculation of sweet pepper with the endophytic entomopathogenic fungi *Beauveria bassiana* ARSEF 3097 and *Akanthomyces muscarius* ARSEF 5128 on the olfactory behaviour and life history of the tobacco peach aphid *Myzus persicae* var. *nicotianae*. First, dual choice experiments were performed to investigate olfactory responses when aphids were given the choice between inoculated and control plants, with and without allowing them to contact the plants. Next, effects of endophytic colonization on the development and longevity of *M. persicae* were studied in clip cage experiments.

Results

Inoculation of sweet pepper plants with endophytic entomopathogenic fungi elicited a positive response of *M. persicae* var. *nicotianae* towards sweet pepper plants, especially when plants were inoculated with *A. muscarius* ARSEF 5128. However, results also demonstrated significantly reduced aphid longevity and reproduction on plants inoculated with *A. muscarius* ARSEF 5128.

Conclusion

Overall, these results indicate the potential of endophytic entomopathogenic fungi in biological pest control by creating dead-end trap crops to which the pest insects are attracted, but survival of the offspring is impaired. Future research with greenhouse experiments is needed to assess its potential to control aphids in real-world conditions.

Key words: *Myzus persicae* var. *nicotianae*, *Capsicum annuum*, entomopathogenic fungi, endophytic colonization, biological pest control

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