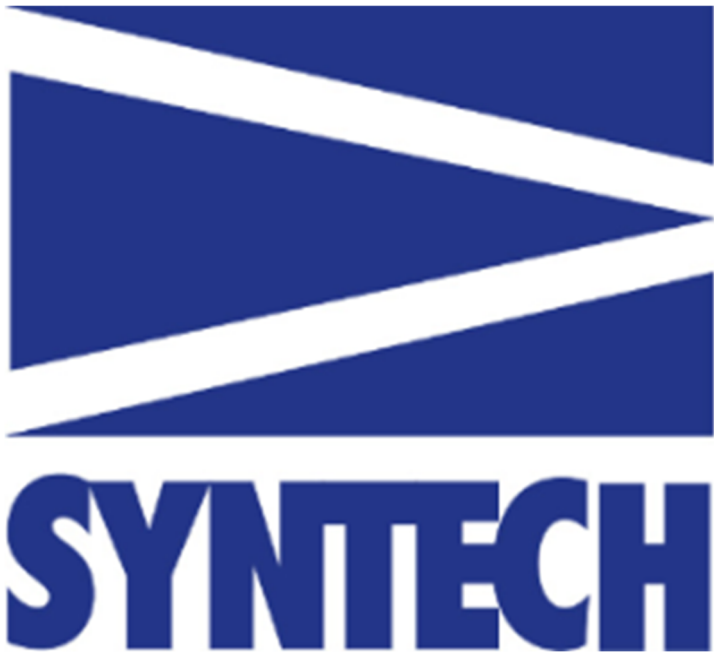
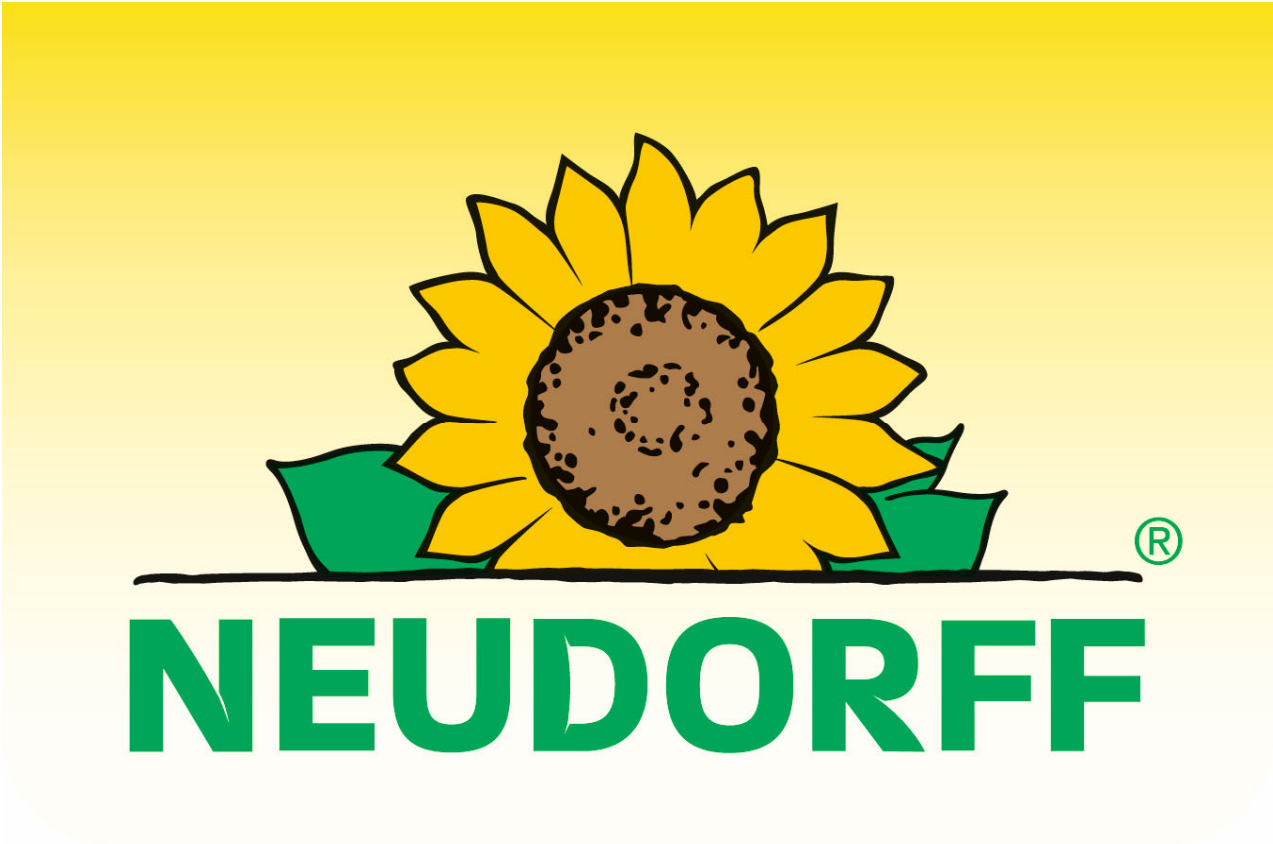




Symposium on Insect-Plant relationships  
Book of Abstracts  
August 4-8, 2024  
Haus Neuland, Bielefeld, Germany



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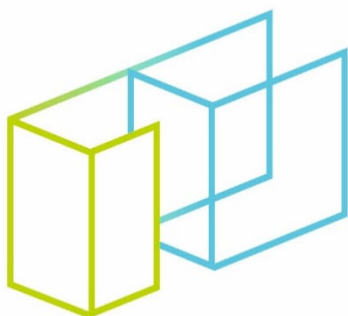




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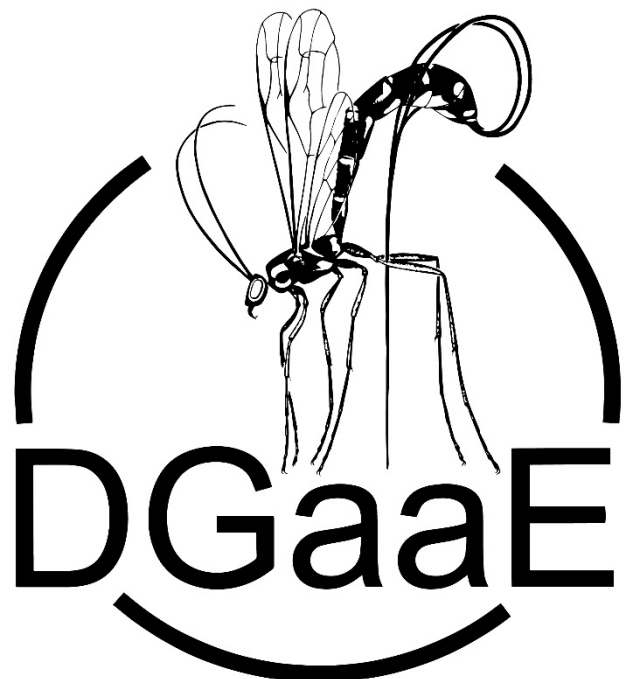


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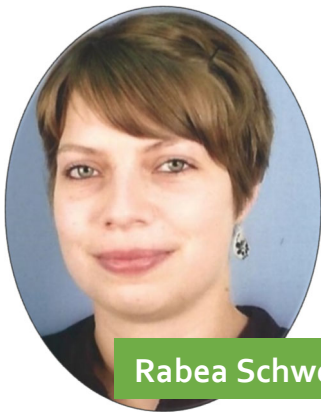


# Welcome to SIP 2024 in Bielefeld, Germany

We are honoured to host the 18th Symposium on Insect-Plant relationships in Bielefeld from 4th to 8th of August 2024. The Symposium on Insect-Plant relationships has a long tradition, is held every three years and deals with various aspects of insect-plant interactions, extending also to other interaction partners. In 2024, it will be for the second time in Germany, after having been hosted in Berlin 20 years ago. This year's conference will take place at the foot of the Teutoburg forest in "Haus Neuland", in the middle of a beautiful countryside, allowing you to enjoy the nature before and after the sessions. In total, 166 participants have signed up, coming from 19 different countries (in terms of current work place). We are looking forward to meeting you in Bielefeld, hear the newest results on exciting insect-plant relationships, discuss fascinating research, enjoy meeting old and new friends, and welcome everybody to the SIP family!

Safe travels and see you soon!

The organising committee



Rabea Schweiger



Christine Fiebig



Caroline Müller

# How to get to Haus Neuland?

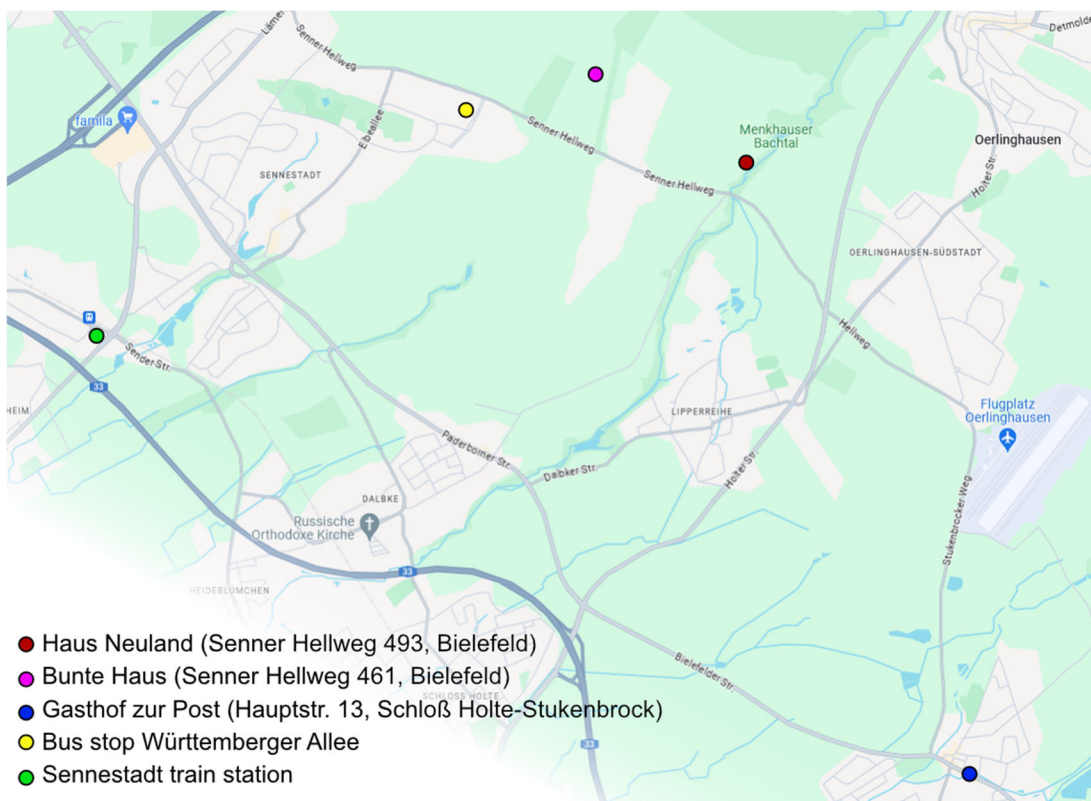
Our public transport companies are using the holiday period to carry out necessary construction work on the rail network. The following route is currently the most favourable:

On arrival at Bielefeld main station, take the regional train (RE) in the direction of Paderborn, platform 8. The train departs hourly (for example 12:43, 13:43, 14:43, 15:43, 16:43) and travels to Bielefeld-Sennestadt (3 intermediate stops). From Bielefeld-Sennestadt station shuttle buses will take you directly to Haus Neuland. These will depart on Sunday, 4<sup>th</sup> of August 2024 at: 13:00, 14:00, 15:00, 16:00 and 17:00. If you arrive at a different time, please register your need for a shuttle via e-mail to [info@haus-neuland.de](mailto:info@haus-neuland.de) or call the reception of Haus Neuland at the following telephone number: +49 5205 91 26 10

There is an alternative, less comfortable way which includes taking the tram line 1 (direction: Brackwede) from Bielefeld central station to Adenauerplatz (4 intermediate stops). From there, take the bus-line 135 from the bus-stop “Kunsthalle” to “Württemberg Allee” (ca. 45 minutes bus ride). From here, it is a 2.3 km walk to Haus Neuland.

If you stay at “Bunte Haus” or “Gasthof zur Post”

Some participants will stay at the hotels “Bunte Haus” or “Gasthof zur Post”. Participants will be taken to Haus Neuland daily by a shuttle bus from Monday – Thursday (05.08.-08.08.) at 07:00 am (Gasthof zur Post) and 07:20 am (Buntes Haus). Likewise, a shuttle bus will pick up all participants from Haus Neuland at 9:30 pm daily from Sunday – Wednesday (04.08.-07.08.) and bring you to “Buntes Haus” and “Gasthof zur Post”. If you miss one of the shuttle rides, please contact the reception of Haus Neuland (Tel: +49 5205 91 26 10)



# Excursions & Workshop



© Caroline Müller

Guided plant excursion to the Ochsenheide and surrounding Teutoburg forest

The Ochsenheide is one of the most valuable nature protection areas in Bielefeld with about 120 plant species, including threatened species.

**Getting there:** 16:15 bus transfer from Haus Neuland

**Start - End:** 17:00 – 18:30

**After:** Bus transfer to conference dinner or back to Haus Neuland



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Guided tour through the Sparrenburg Castle

The castle is the most famous landmark of Bielefeld. You can enjoy a wonderful panoramic view over Bielefeld. The tour guides through the casemates of the castle.

**Getting there:** 16:15 bus transfer from Haus Neuland

**Start - End:** 17:00 – 18:30

**After:** Bus transfer to conference dinner or back to Haus Neuland

Insect safari in the surrounding of Haus Neuland

The region around Haus Neuland will be explored by the participants. The guided tour will focus on insect species and their habitats.

**Getting there:** Starts directly at Haus Neuland

**Start - End:** 16:15 – 17:45

**After:** Bus transfer to conference dinner.



© Claudia Quirini-Jürgens

Guided Tour through Zoo Olderdissen

“Heimat-Tierpark Olderdissen” is a zoo embedded in the Teutoburg forest with various Central European animal species. A guided tour focussing on nature/wildlife conservation is offered.

**Getting there:** 16:15 bus transfer from Haus Neuland

**Start - End:** 17:00 – 18:30

**After:** Bus transfer to conference dinner or back to Haus Neuland



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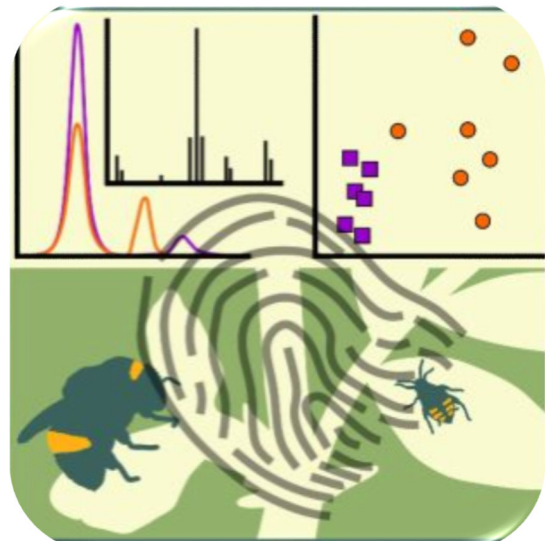
## Workshop Eco-Metabolomics

The complementary nature of ecology and metabolomics is becoming increasingly apparent. Here, we will explore strategies and tools used to combine both fields and improve our understanding of plant-environment interactions. General strategies, analytical techniques, processing programmes and the concept of predictive metabolomics will be discussed.

**Getting there:** Takes place at Haus Neuland

**Start - End:** 16:15 – 17:45

**After:** Bus transfer to conference dinner



© Saskia Karolak, Rabea Schweiger



# Scientific programme



**17:45 – 18:45**      **Opening note**  
Chair                    Robert Junker (Marburg/DE)  
Speaker                Renee M. Borges (Bangalore/IN)  
Topic                    Chemodiversity, genetic diversity and phenotypic plasticity: Implications for plant-insect food webs under climate change

**From 18:45 on**      **Welcome reception**



**6:30 – 8:45**            **Breakfast**

**8:45 – 12:15**        **Session Environmental Change**

Chair                    Anurag Agrawal (Ithaca/US)

8:45 – 9:30            Effects of climate change on insects  
Keynote                Rieta Gols (Wageningen/NL)

9:30 – 9:45            Urban heterogenous environment and trait multi-functionality shape the evolution of white clover  
Speaker                Tomoki Ishiguro (Hokkaido/JP)

9:45 – 10:00         We've always done it this way: does grass cover crop management influence arthropod diversity and abundance in long-term drought?  
Speaker                Alice Casiraghi (Moncada/ES)

10:00 – 10:15        Flowering and insect emergence after a warmer winter and an earlier spring  
Speaker                Andrey Malyshev (Greifswald/DE)

**10:15 – 11:00**        **Coffee break**

11:00 – 11:15        The effect of climate change on plant-herbivore interaction along elevation gradients  
Speaker                Sergio Rasmann (Neuchâtel/CH)

11:15 – 11:30        Climate warming disrupts conifer phenological defenses against a boreal outbreaking pest  
Speaker                Emma Despland (Montreal/CA)

11:30 – 11:45        Attenuation of the wound-induced jasmonate burst in the C3 plant *Arabidopsis thaliana* grown at eCO<sub>2</sub>  
Speaker                Jacquie Bede (Montreal/CA)

11:45 – 12:00 Speaker	The impact of salinity on plant-insect interactions Sahil Pawar (Pennsylvania/US)
12:00 – 12:15 Speaker	Resistance to the whitefly <i>Bemisia tabaci</i> is compromised in salinity-stressed <i>Capsicum</i> plants Lotte Caarls (Wageningen/NL)
<b>12:15 – 13:45</b>	<b>Lunch</b>
<b>13:45 – 16:15</b> Chair	<b>Session Sequestration, Pharmacophagy and Addicts</b> Beata Gabryś (Zielona Góra/PL)
13:45 – 14:30 Keynote	Plant toxins as drivers of insect-plant coevolution Georg Petschenka (Stuttgart/DE)
14:30 – 14:45 Speaker	Plant metabolites modulate chemical defence, social interactions and fitness in a sawfly Pragya Singh (Bielefeld/DE)
14:45 – 15:00 Speaker	Glucosinolates at the next trophic level: effects of aphid-sequestered compounds on a ladybird predator Duc Tam Mai (Jena/DE)
15:00 – 15:15 Speaker	Cardenolide uptake, metabolization and excretion by non-adapted <i>Erysimum</i> herbivores Tobias Züst (Zurich/CH)
<b>15:15 – 15:45</b>	<b>Coffee break</b>
15:45 – 16:00 Speaker	Divergence in toxin composition of two subspecies of swamp milkweed and the impact on their specialized herbivores Paola Rubiano-Buitrago (Ithaca/US)
16:00 – 16:15 Speaker	The Na,K-ATPase $\beta$ -subunit influences cardenolide distribution in the body fluids of a cardenolide-adapted insect Marlene Herbertz (Hamburg/DE)
<b>16:15 – 16:45</b>	<b>General Assembly</b>
<b>16:45 – 18:45</b>	<b>Poster Session (odd numbers)</b>
<b>From 18:45 on</b>	<b>Dinner</b>

6:30 – 8:45

**Breakfast**

8:45 – 12:15

**Session Molecular Mechanisms and Eco-omics**

Chair

Georg Petschenka (Stuttgart/DE)

8:45-9:30

Molecular mechanisms underlying agroecological pest control as practiced by smallholder farmers in sub-Saharan East Africa

Keynote

Meredith Christine Schuman (Zurich, CH)

9:30 – 9:45

Deciphering the defense strategies of wild mungbean against *Spodoptera litura*

Speaker

Hieng-Ming Ting (Taipei/TW)

9:45 – 10:00

Prediction and validation of terpene synthase functions in *Tanacetum vulgare*

Speaker

Marvin Hildebrandt (Bielefeld/DE)

10:00 – 10:15

Natural rubber reduces herbivory and alters the microbiome below ground

Speaker

Meret Huber (Mainz/DE)

10:15 – 11:00

**Coffee break**

11:00 – 11:15

Loss of olfaction through mutagenesis of the olfactory co-receptor Orco impairs foraging and natural enemy avoidance of *Pieris brassicae* caterpillars

Speaker

Alexander Haverkamp (Wageningen/NL)

11:15 – 11:30

Oviposition-induced plant volatiles prime undamaged rapeseed defences against upcoming herbivores.

Speaker

Pius Otto (Gif-sur-Yvette/FR)

11:30 – 11:45

Getting the big picture: studying the ecological consequences of producing antimicrobial defences during root herbivory

Speaker

Axel Touw (Großbeeren/DE)

11:45 – 12:00

Molecular mechanisms of organ-specific steroidal glycoside diversity in *Solanum dulcamara* affecting insect-plant interactions: a bittersweet story

Speaker

Nicole M. van Dam (Großbeeren/DE)

12:00 – 12:15

A sweet starter? *Solanum dulcamara*'s wound nectar - an inheritable and polymorphic trait

Speaker

Anke Steppuhn (Stuttgart/DE)

12:15 – 13:45

**Lunch**

13:45 – 16:45

**Session Spatio-temporal Dynamics**

Chair

Sharon Zytynska (Liverpool/GB)

13:45 – 14:30

Convergence and divergence in the diversity of insects feeding on a toxic plant

Keynote

Anurag Agrawal (Ithaca/US)

14:30 – 14:45

Vertical stratification of leaf physical traits exerts bottom-up pressures on insect herbivory in a sugar maple temperate forest

Speaker

Mahsa Hakimara (Montreal/CA)



SCIENTIFIC PROGRAMME | TUESDAY, 6 AUGUST

- 14:45 – 15:00  
Speaker Plant defense under Arctic light conditions: Plants vs pests  
Clabe Wekesa (Jena/DE)
- 15:00 – 15:15  
Speaker Quantifying functional diversity-index via UV-Vis spectral identity: A novel route to compound dissimilarity matrix  
Kruthika Sen Aragam (Stuttgart/DE)
- 15:15 – 15:45 Coffee break**
- 15:45 – 16:00  
Speaker Ecological conflicts of floral defenses in the high Andean shrub *Bejaria resinosa* (Ericaceae)  
Andre Kessler (Ithaca/US)
- 16:00 – 16:15  
Speaker Understanding the effects of biogeography and domestication on a facultative ant-plant mutualism in an ancient alien species  
Pooja Nathan (Toronto/CA)
- 16:15 – 16:30  
Speaker Immature leaves are the dominant volatile sensing organs of maize  
Lei Wang (Bern/CH)
- 16:30 – 16:45  
Speaker Plant induced defenses promote transgenerational effects including intraclutch cannibalism in Colorado potato beetle  
Moshe Zaguri (Rishon LeTsiyon/IL)
- 16:45 – 18:45 Poster Session (even numbers)**
- From 18:45 on Dinner**



SCIENTIFIC PROGRAMME | WEDNESDAY, 7 AUGUST

- 6:30 – 8:45 Breakfast**
- 8:45 – 12:15 Session Vascular Systems**  
Chair Rieta Gols (Wageningen/NL)
- 8:45 – 9:30  
Keynote Aphids as sensors: exogenous application of allelochemicals and aphid probing behavior  
Beata Gabryś (Zielona Góra/PL)
- 9:30 – 9:45  
Speaker Plant part-specific niche construction by the specialist aphid *Sitobion avenae* on wheat  
Rabea Schweiger (Bielefeld/DE)
- 9:45 – 10:00  
Speaker A comparative proteomic and metabolomic study of phloem sap from sweet orange and lemon trees infested by *Trioza erytreae*  
Tomás Magalhães (Bragança/PT)

10:00 – 10:15	Comparative osmoregulation strategies and host plant adaptation in <i>Bemisia tabaci</i>
Speaker	Osnat Malka (Jerusalem/IL)
<b>10:15 – 11:00</b>	<b>Coffee break</b>
11:00 – 11:15	Identifying and characterizing members of the glycoside hydrolase family 13 with sucrose isomerase activities in <i>Bemisia tabaci</i>
Speaker	Dor Wintraube (Jerusalem/IL)
11:15 – 11:30	Mealybug honeydew: how does its composition change under elevated CO <sub>2</sub> and water limitation?
Speaker	Christine Becker (Geisenheim/DE)
11:30 – 11:45	Rhizobacteria-induced transcriptomic changes to aphids on cereal crop barley
Speaker	Crispus Mbaluto (Liverpool/GB)
11:45 – 12:00	Endosymbionts modulate virus effects on aphid-plant interactions
Speaker	Patricia Sanches (Zurich/CH)
12:00 – 12:15	Soil type has drastic influence on corn leaf aphid performance
Speaker	Anju Poudel (Pennsylvania/US)
<b>12:15 – 13:45</b>	<b>Lunch</b>
<b>13:45 – 15:30</b>	<b>Session Inorganic versus Organic Plant Defences</b>
Chair	Meredith Schuman (Zurich, CH)
13:45 – 14:30	Down-to-earth defence: how grasses use silicon against insect herbivores in a multitude of ways
Keynote	Scott Johnson (Richmond/AU)
14:30 – 14:45	Elemental defense: harnessing zinc for enhanced rice protection against <i>Cnaphalocrocis medinalis</i>
Speaker	Ya-Fen Lin (Taipei/TW)
14:45 – 15:00	Inorganic versus organic defences of plants against herbivorous insects: insights from hyperaccumulators
Speaker	Rocky Putra (Bielefeld/DE)
15:00 – 15:15	Elucidating the defensive functions of calcium oxalate crystals (raphides) in <i>Vitis</i>
Speaker	Carolyn Graham (East Lansing/US)
15:15 – 15:30	Arbuscular mycorrhizal fungi promote insect herbivore performance, despite increasing anti-herbivore silicon defences in maize
Speaker	Ramalka H Kasige (Richmond/AU)
<b>15:30 – 16:00</b>	<b>Coffee break</b>
<b>16:00</b>	<b>Departure to excursions (see “Excursions”)</b>
<b>17:45</b>	<b>Departure to conference dinner</b>
<b>19:00</b>	<b>Conference dinner</b>

**6:30 – 8:45**

**Breakfast**

**8:45 – 11:30**

**Session Crop Plants and Agriculture**

Chair

Scott Johnson (Richmond/AU)

8:45 – 9:30

Crop plants and agriculture: Insect-plant relationships from an applied perspective

Keynote

Sharon Zytynska (Liverpool/ENG)

9:30 – 9:45

Digging the diversity of wild potatoes – metabolomics reveals species- and tissue-specific metabolites promoting resistance against leaf beetles

Speaker

Torsten Meiners (Berlin/DE)

9:45 – 10:00

Flower-derived arthropod eDNA reveals community diversity, species abundances and ecological interactions – a case study in bee pollinators

Speaker

Arndt Schmidt (Trier/DE)

10:00 – 10:15

Unveiling the role of cover crop mixtures: implications for maize resistance to pests and pathogens

Speaker

Alison Jennings (Pennsylvania/US)

**10:15 – 11:00**

**Coffee break**

11:00 – 11:15

Can arthropod pests evolutionary adapt to systemic resistance induced by the microbial symbionts of their host plants?

Speaker

Arjen Biere (Wageningen/NL)

11:15 – 11:30

Dual function of omnivorous predators in biocontrol: as bodyguards and plant defence vaccinators

Speaker

Nina Xiaoning Zhang (Wageningen/NL)

**11:30 – 12:15**

**Closing note**

Chair

Renee M Borges (Bangalore/IN)

Speaker

Robert Junker (Marburg/DE)

Topic

Microbes modulate plant performance and responses to global change

**12:15 – 13:45**

**Lunch**

**Departure**

# Oral Presentations



**Keynote: Chemodiversity, genetic diversity and phenotypic plasticity: Implications for plant-insect food webs under climate change**

Renee M. Borges

*Indian Institute of Science, Bangalore, India*

The axes of chemodiversity and its causes are many. The scales at which chemodiversity is measured are also varied. Chemodiversity may arise due to genetic or epigenetic factors and plants may also exhibit phenotypic plasticity. Chemodiversity may be measured at the whole plant level and compared between individual plants, at the organ level (stems, leaves, roots, flowers and fruit), or at the ontogenetic level of these organs and compared between individual plants. Chemodiversity may also be measured within a plant and between modules of the same organs, e.g. between leaves of one plant module and another. Variation within a plant and between modules may arise due to plasticity and local resource flows, it may also arise from somatic genetic mutations in different modules. The latter has been little explored in terms of generation of local intra-plant chemodiversity. Chemodiversity may also arise due to localised herbivory and/or pathogen attacks and these effects may be transient. Feedback between and within plant organs and modules can also result in diverse chemistry. Therefore, the chemotype appears to be a shifting phenotype and the degree of its variance needs to be adequately captured. Similarly, the degree of invariance also requires documentation. For example, are some chemical compounds highly variable within floral scent and are some quite invariant in terms of their coefficients of variation? Perhaps more attention needs to be paid to the invariant compounds rather than those that vary considerably in order to decipher the nature of the signals and cues in within plant/between plant or in plant–insect interactions. This keynote address will describe the axes of chemodiversity and suggest interesting questions that arise from such investigations. These axes are also likely to shift in predictable or unpredictable ways under the impact of climate change. The consequences for insect–plant food webs will be explored.

**Keynote: Effects of climate change on insects**

Rieta Gols

*Wageningen University & Research, Wageningen, Netherlands*

Evidence has shown that the decline in biodiversity, including that of insects, is prominently driven by anthropogenic global change. This is a concern considering the role that insects play in ecosystems by providing vital services such as pollination, biological control, food for higher trophic levels, and decomposition. Anthropogenic global change is multifaceted and includes factors such as climate change, fragmentation and loss of habitats, agricultural intensification, and pollution. Here, I will focus on the effects of climate change. It is characterized by both deterministic (longer-term), and stochastic (short-term) phenomena. Multidecadal warming is described by a slow rise in mean surface temperature, whereas at shorter timescales (days, weeks) there has been an increase in the frequency, duration, and intensity of climatic extremes, such as heatwaves. The effects of gradual warming have received relative more attention in the scientific literature than effects of extreme climatic events. However, exposure to climate extremes, may strongly impact small ectothermic organism, such as insects, in particular, when their ability to move and seek refuge during these events is limited. Moreover, climatic extremes such as heatwaves are often accompanied by other extreme event such as drought, and or extreme rainfall, which can exacerbate the perceived stress levels. In this presentation, I will give an overview of how insect can be affected by the various aspects of climate change.

## Urban heterogenous environment and trait multi-functionality shape the evolution of white clover

Tomoki Ishiguro<sup>1</sup>, Marc Johnson<sup>2</sup>, Shunsuke Utsumi<sup>1</sup>

<sup>1</sup>Hokkaido University, Sapporo, Japan

<sup>2</sup>University of Toronto Mississauga, Mississauga, Canada

The threat of urbanization for biodiversity is a global concern due to the profound effects on environmental changes. In recent years, urban environmental change has been reported to impact the evolution of lives in many taxa. However, our understanding toward how urban environments influence evolutionary processes and outcomes is still limited. Previous studies on urban evolution have generally applied transects or dichotomic comparisons between urban and rural areas, ignoring the non-linearity of environmental variables arise from urban heterogeneity. Furthermore, many traits are often multi-functional, and the relative importance of each function of a multi-functional trait depends on the surrounding environment. Such multi-functionality of traits, coupled with complex spatial variation in environmental gradients from urban to rural areas, could make the relationships between urbanization and trait evolution highly complex.

In this study, we focused on the evolution of white clover. It has a Mendelian polymorphism for the production of hydrogen cyanide (HCN) and its components (cyanogenic glycosides and the hydrolytic enzyme linamarase), which are multi-functional traits, making it an ideal species for examining how plants evolve and adapt to complex multivariate urban environmental heterogeneity.

To comprehensively elucidate the spatial pattern of evolutionary traits in urban environments, we collected 3299 white clover plants from 122 populations across Sapporo, Japan. Then, we surveyed the relationship between the plants producing HCN (and its components) and the spatial variation in environmental factors such as herbivory, sky openness, impervious surface cover, snow depth, and temperature. The heterogeneity of the urban landscape led to complex spatial variation in environmental factors. Among them, herbivory, sky openness, and impervious surface cover strongly influenced the frequency of plants producing HCN in populations. Notably, impervious surface cover was associated with the frequency of plants producing cyanogenic glycosides, while herbivory pressure was not. As a result, the cyanogenic glycoside frequency exhibited a more pronounced trend along urban–rural gradient than the frequency of HCN. Thus, the predicted spatial distributions of HCN and cyanogenic glycosides were inconsistent. These findings highlighted that urban landscape heterogeneity and trait multi-functionality shape mosaic-like spatial distribution of evolutionary traits.

## **We've always done it this way: Does grass cover crop management influence arthropod diversity and abundance in long-term drought?**

Alice Casiraghi<sup>1</sup>, Víctor Moreno-González<sup>2</sup>, Alberto Urbaneja<sup>1</sup>, Nicolás Pérez Hidalgo<sup>1</sup>, César Monzó Ferrer<sup>1</sup>

<sup>1</sup>IVIA - Valencian Institute for Agricultural Research, Moncada, Spain

<sup>2</sup>Biodatev, León, Spain

Cover crops are green infrastructures that are generating intense interest in the European Union because they can enhance climate change mitigation, improve soil quality, and promote biodiversity of the agroecosystem.

However, our lack of knowledge about interactions among cover crops, arthropods and crops of interest generates doubts and controversies on their utility and management, especially in global climate-induced challenging situations such as the long-term drought that some areas of the Mediterranean are experiencing. It is common knowledge among farmers and agronomists that grass strips in alleyways must be managed by cutting at least once yearly to maintain the desired plant communities and avoid workers discomfort during harvest. Yet, little is known about the consequences of this practise on their biodiversity.

In this work, we study how two different types of grass (*Festuca arundinacea*) cover crop management – traditional yearly cut or not cut – induce differences in the composition and abundance of the arthropod communities inhabiting them in a rainfall deficit context.

A survey spanning an entire year inside a mandarin orchard (Pego, Alicante, Eastern Spain) tested sown cover crops in a randomized block design. In May 2023, half of the *F. arundinacea* blocks underwent traditional management (one cut in spring), while the other four were left untouched. The species composition, height, and grass coverage were recorded monthly. At the same time, arthropods were also sampled from the grass block central row with a suction device, counted, and identified.

Under actual long-term drought conditions, grass undergoing traditional management suffered from the lack of water, and its growth was limited after one year, while untouched *F. arundinacea* formed tussock-like grass strips.

The different management was mainly reflected in different composition and abundance of arthropods, primarily cover crop phytophagous insects, but also natural. Significantly higher numbers of Auchenorrhyncha and three gramineous aphid species (Sternorrhyncha) were recorded from untouched blocks, Higher mean numbers of parasitoids, as well as ground-dwelling predators of the Staphylinidae family and spiders were recorded in the same treatment.

Our findings emphasize the need for knowledge about plant-arthropod communities determined by long-lasting field experiments to make cautious and money-saving decisions about cover crop and semi-natural habitat management.

## Flowering and insect emergence after a warmer winter and an earlier spring

Andrey Malyshev, Hai Luong Son

*University of Greifswald, Greifswald, Germany*

Species-specific phenological responses of insects and plants to winter warming may result in pollination disruption. Yet experimental approaches where both insects and plants are warmed during their non-growing phases are lacking. We simulated warming for a group of diapausing insects and dormant flowering plants to quantify the insect and plant survival and phenological shifts.

Two solitary bees (*Osmia bicornis* and *Osmia cornuta*) in their cocoon overwintering stage, and one butterfly (*Pieris napi*) in its pupae stage, were placed in chambers where a warm (7°C night / 12°C day) or a cool winter (0° C night /6 ° C day) were simulated from November to February. Seven flowering species (*Pulmonaria officinalis*, *Plantago media*, *Ajura reptans*, *Corydalis solida*, *Muscari neglectum*, *Crocus tommasinianus*, *Scilla bifolia*), commonly pollinated by the insects, were grown in pots and placed in the same climate chambers. In late February all insects and plants were transferred to a greenhouse and kept until emergence and flowering (12°C night/ 18 °C day). A separate group of plants and insects was allowed to overwinter outside in the field, being either brought into the greenhouse in February or in March, simulating an earlier or a later spring onset. Phenology, emergence and flowering success as well as flower and insect size were recorded.

A warmer winter advanced bee emergence but did not affect butterfly emergence. Flowering timing was advanced by winter warming in earlier flowering species but did not affect later flowering species. The earlier spring onset delayed bee emergence but did not affect butterfly emergence. Flowering was delayed due to the earlier spring onset in the earlier flowering species. The variation in flowering timing among plants increased in the warmer treatments. Percent emergence was reduced in the butterfly after winter warming while flower size was reduced in one species in the earlier spring.

The results point to a projected shift in phenological ranking in both plants and insects due to species-specific sensitivity to winter and spring warming. Generalist pollinators will likely have a different “menu” of flowering species during their peak emergence, with the effects of different food sources on insect fitness requiring further study. Plant and insect species with late flowering and emergence timings may be at a higher risk of mortality with continued warming.

## **The effect of climate change on plant-herbivore interaction along elevation gradients**

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A current significant challenge in ecology involves predicting the responses of communities and ecosystems to global climate change. The impact of global change, beyond directly affecting plant communities and their functioning, it can also modify interactions among species. For example, climate warming may lead to shifts in organism distributions, with varying rates of movement among different species, ultimately causing alterations in the composition and operation of ecological communities. These effects are often overlooked in forecasts regarding the impact of climate change on biodiversity. I will demonstrate that experimental translocations can be utilized to explore the consequences of uneven upslope migration in alpine plants and their insect herbivores on community interactions. Subsequently, I will elaborate on how these reorganized trophic interactions will play a crucial role in driving changes in plant communities under anticipated future climate conditions.

## Climate warming disrupts conifer phenological defenses against a boreal outbreaking pest

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Phenological shifts, induced by global warming, can lead to mismatch between closely interacting species or even form new matches. The eastern spruce budworm, *Choristoneura fumiferana*, an important outbreaking insect defoliator of the boreal forest, mainly feeds on balsam fir, *Abies balsamea*, which has historically been well synchronized with the insect. But as climate change pushes the northern range limit of the budworm further north into the boreal forest, the highly valuable black spruce, *Picea mariana* is suffering increased defoliation during the current outbreak. Budworm exploit a narrow window of opportunity in spring by feeding on expanding buds. Improved synchronization of this window on black spruce could explain the current range expansion of budworm onto black spruce.

We first test how synchrony between budworm emergence and budbreak of the two host plants influences budworm performance with a 2-year field experiment rearing budworm larvae on both hosts at different lags with tree budburst phenology. We next tested the hypothesis that rising temperatures can lead to an improved match between the budworm and black spruce through differential phenological advancement with a 3-year experiment rearing budworm on both hosts in temperature enhancement mesocosms.

Results show that, on both hosts, late-emerging larvae suffer both high mortality and poor development, presumably linked to decreasing foliar quality during the growing season. By contrast, early emerging larvae show good growth but high mortality relative to synchronized larvae. Further lab experiments suggest that the survival of early-emerging larvae is constrained by their ability to enter a needle and that this critical step in the life-cycle depends on needle toughness.

Mesocosm results show that under higher temperature regimes, eastern spruce budworm will be as successful on black spruce as on balsam fir, as black spruce budburst becomes better synchronized with the insect's emergence from diapause. This could lead to critical changes in outbreak dynamics and severity with important ecological state shifts at the landscape level.

## Attenuation of the wound-induced jasmonate burst in the C3 plant *Arabidopsis thaliana* grown at eCO<sub>2</sub>

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Jasmonates phytohormones play an important role in plant resistance against attack by necrotrophic pathogens and chewing herbivores. Our lab has found that the jasmonate burst in response to mechanical damage is attenuated in C3 plants of *Arabidopsis thaliana* grown at elevated CO<sub>2</sub> (eCO<sub>2</sub>). Redox and transcriptomic studies have given us some insight into potential underlying connections between eCO<sub>2</sub> and plant defensive hormone biosynthesis.

## The impact of salinity on plant-insect interactions

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The progressive salinization of agricultural land poses a significant threat to global crop productivity. Concurrently, the presence of insect herbivores adds additional pressures, compounding agricultural losses. Despite extensive research on the impact of salinity on plant physiology, very few studies try to understand its implications for plant-insect interactions. In this study, we show that salt-stressed tomato plants (*Solanum lycopersicum* cv. Better Boy) are poor hosts for the corn earworm (*Helicoverpa zea*), reducing caterpillar preferences, performances, and moth oviposition. We attribute these observations to reductions in relative leaf water content, leaf total protein content, alterations in plant direct defenses, and ionic toxicity. We shed light on the effect of ionic toxicity on insect behavior and growth, an often-overlooked aspect. Furthermore, we explore the cascading impacts of salinity stress on higher trophic levels, such as on predators and parasitoids. We investigate how insect quality as prey (for predators) or hosts (for parasitoids) changes with plants under salt stress. Additionally, we study indirect plant defenses under salt stress by using volatile organic compound (VOC) analysis and investigate natural enemy recruitment via Y-tube olfactometer assays. Intriguingly, we find that predators are attracted to salt-stressed plants, while parasitoids are attracted to plants without salt stress. The effects of salt-stress are seen on plants, their insect herbivores, and their natural enemies.



## Resistance to the whitefly *Bemisia tabaci* is compromised in salinity-stressed *Capsicum* plants

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Climate change has profound effects on crop production, for example through salt intrusion in deltas. In addition, pest pressure will change. How plants cope under double stress conditions is dependent on pest species, environmental conditions, and genotype, and for many plant-insect interactions, knowledge on this is lacking. In this study, we aimed to identify pepper (*Capsicum* sp.) accessions with resistance to the whitefly *Bemisia tabaci*, and we studied how this resistance was affected by salinity stress. To do this, we grew 25 *Capsicum* accessions under salinity treatment, and measured *Bemisia tabaci* adult survival and oviposition. To study the effects of salinity stress on plants, plant weight and height were measured. Under salinity stress, growth of most accessions was inhibited, and Na<sup>+</sup> accumulated in the shoot. We identified four accessions with increased resistance to whitefly, exhibited as increased adult mortality and reduced oviposition. However, in all plants that had experienced a salt stress, whitefly survival and oviposition increased, essentially nullifying resistance in salt-stressed plants. We found that accumulation of the phytohormone jasmonic acid was reduced compared to whitefly-infested plants without salt, possibly explaining the effect on whitefly resistance. In conclusion, for both salt tolerance and whitefly resistance there is genetic variation available that may be used to breed new cultivars with improved performance. Studying the interaction between both stresses may result in tools to grow plants that are stable resistant in changing conditions.

## **Keynote: Plant toxins as drivers of insect-plant coevolution**

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Herbivorous insects are exposed to a wide variety of toxic compounds while feeding on their host plants. Remarkably, many insects can not only cope with plant toxins, but also sequester them for defense against predators. Thus, plant toxins mediate biotic interactions across trophic levels and drive the plant-insect coevolutionary arms race by selecting for a variety of traits ranging from simple molecular adaptations to complex behaviors. Although sequestration is widespread, we still lack a general understanding of several central pillars of the sequestration syndrome, including mechanisms of transport and selectivity, imposed physiological costs, and evolutionary trajectories. We investigate the sequestration of cardiac glycosides (cardenolides) in danaine butterflies and milkweed bugs at multiple scales. Specifically, we aim to understand the molecular and physiological adaptations underlying sequestration and their implications for the evolution of biotic interactions. We found that sequestration of plant toxins selects for resistance mechanisms that are distinct from those required to consume a toxic diet. Moreover, cardiac glycoside resistance that evolved in accordance with ancient host-plant associations may serve as a preadaptation, facilitating specialized sequestration of the same class of toxins from phylogenetically unrelated plants. Consequently, predators that select for sequestration can drive the evolution of specific resistance traits and novel host-plant associations. While cardiac glycoside resistance in sequestering insects is well understood, it is unclear how cardenolides are transported across the gut epithelium. We suggest that quantitative differences between sequestering and non-sequestering insects may not be mediated by properties of the gut epithelium alone, but likely involve additional mechanisms such as differential processing of toxins in the gut lumen or excretion. Finally, theory predicts that defense by sequestered toxins comes at a physiological cost and compromises individual fitness components. In milkweed bugs, however, we found that sequestered toxins reduced developmental time and increased lifespan, suggesting that sequestered toxins serve as physiological assets in specialized insects. In summary, we have uncovered diverse mechanisms by which predators selecting for sequestration interfere with insect-plant coevolution and revise established paradigms of the sequestration syndrome.

## **Plant metabolites modulate chemical defence, social interactions and fitness in a sawfly**

Pragya Singh, Leon Brüggemann, Caroline Müller

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Plant-insect interactions encompass more than just nutrition, as insects utilise plant metabolites for various purposes, including chemical defence and pheromone synthesis. The turnip sawfly, *Athalia rosae*, obtains neo-clerodane diterpenoids (hereafter “clerodanoids”) from non-host plant species like *Ajuga reptans* through pharmacophagy. Adult sawflies can also acquire clerodanoids from conspecifics via agonistic social interactions such as nibbling. We demonstrated that pharmacophagy of clerodanoids conferred protection against predation, even when these metabolites were obtained indirectly through conspecifics. Sawflies lacking direct access to clerodanoids experienced reduced predation risk when grouped with defended conspecifics, indicating that the social niche in which individuals live can impact their survival. Moreover, we observed substantial variation in clerodanoid amounts in both wild-caught and lab-reared individuals, underscoring the significance of individual differences in natural populations. Network analysis revealed that sawfly groups in which either all or some individuals had prior clerodanoid access exhibited a higher frequency of social interactions compared to those without such access. Additionally, the frequency of social interactions depended on both the clerodanoid status of the focal individual and that of other conspecifics. However, clerodanoid acquisition also came with costs, as evidenced by a shortened lifespan observed in adults with prior clerodanoid access when paired with individuals lacking such access. This potentially resulted from asymmetric clerodanoid access leading to increased social interactions, particularly of an agonistic nature. These findings highlight the multifaceted consequences of pharmacophagy on chemical defence, social networks and fitness in sawflies, which shape their individual niches and impacts ecological and social dynamics within insect populations.

## Glucosinolates at the next trophic level: Effects of aphid-sequestered compounds on a ladybird predator

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Plants from the Brassicaceae family defend themselves against herbivores by producing glucosinolates, which give rise to toxic breakdown products. Some herbivores, such as the phloem-feeding cabbage aphid *Brevicoryne brassicae*, have in turn evolved mechanisms to cope with these plant toxins, including sequestration for their own defense against predators. Several studies have described cases in which predators are exposed to glucosinolates when they ingest this toxic aphid species, and their development and survivorship are negatively affected. However, the biochemical mechanisms underlying the toxic effects are still unclear. Glucosinolate breakdown products, such as isothiocyanates (ITCs), are believed to cause toxicity by forming conjugates with critical cellular molecules such as glutathione and proteins. However, there is little direct evidence of this for predators of herbivores. In this study, I investigated how a predatory ladybird beetle metabolizes the toxic ITCs from its glucosinolate-sequestering aphid prey. We discovered a novel mechanism potentially underlying toxicity, where ITCs conjugate an enzyme cofactor in the ladybird's body leading to a cascade of negative consequences, including disruption of amino acid metabolism, defects in insect cuticle development, higher susceptibility to an entomopathogenic fungus, and significant damage to DNA. Simulation of the mode of action of ITCs by RNAi disrupted cofactor availability and phenotypically mimicked the effects of toxic ITCs. Such information on the mechanism of ITC action allows us to better understand the impact of plant toxin sequestration in a multitrophic context.

## Cardenolide uptake, metabolism and excretion by non-adapted *Erysimum* herbivores

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Plants in the Brassicaceae genus *Erysimum* evolved the ability to produce cardenolide compounds as novel chemical defences, which are co-expressed with ancestrally conserved glucosinolates by most *Erysimum* species. As a result of this novel trait, many herbivores with specialized adaptations for coping with glucosinolates no longer use *Erysimum* as a host. Nonetheless, some herbivores remain associated with *Erysimum*, despite apparently lacking specialized adaptations for coping with the novel cardenolide defence.

Using a community of three *Erysimum*-associated herbivores, we found surprising diversity in the strategies for uptake, metabolism and excretion of the novel cardenolides. The diamondback moth *Plutella xylostella* occasionally feeds on the leaves of *E. cheiranthoides*, and during feeding not only contains high levels of cardenolides in its gut, but reaches physiologically inhibiting concentrations of cardenolides in its haemolymph. While it is unknown how *P. xylostella* avoids inhibition of its Na<sup>+</sup>/K<sup>+</sup>-ATPase as the main target of cardenolides, cardenolide profiles of caterpillar frass differ from intact leaf material by a significant reduction of di-glycosylated cardenolides, suggesting at least some catabolic activity against cardenolides in the insect gut. In contrast, the mustard aphid *Lipaphis erysimi* accumulates plant cardenolides in its body and excretes them as honeydew largely unchanged, although aphids appear to be susceptible to high concentrations of certain cardenolide compounds in their host plant. Finally, the cabbage bug *Eurydema ornata* commonly feeds on *E. cheiranthoides* and sequesters substantial amounts of glucosinolates in its body, but contains no traces of cardenolides in its body or faeces, indicating pre-ingestive degradation or selective uptake of plant metabolites.

Together, these examples demonstrate how non-adapted herbivores may at least partially tolerate novel plant defences, which is a likely stepping stone for the evolution of more specialized resistance.

## Divergence in toxin composition of two subspecies of swamp milkweed and the impact on their specialized herbivores

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In the study of plant-herbivore coevolution, it is often hypothesized that evolutionary changes in plant secondary metabolites (PSM) contribute to the origin of new plant species and alteration of subsequent interactions with herbivores (1). To retrospectively address this hypothesis from a mechanistic point of view, we describe the PSM content of two subspecies of swamp milkweed (*Asclepias incarnata* *incarnata* and *A. incarnata* *pulchra*) and their specialized insects (2). The phytochemical comparison is carried out through a targeted spectrometric analysis of their cardenolides, i.e., defensive toxins of *Asclepias*, in extracts from five different plant tissues (seeds, roots, stems, leaves and latex).

Overall, we found 30 cardenolide-like compounds across samples. The cardenolides glucopyranosyl frugoside and its aglycone, frugoside, were prevalent in both species and all tissues, most predominantly in the seeds. Less abundant cardenolides in roots and latex differentiated subspecies, with *A. incarnata* *pulchra* displaying higher cardenolide diversity. Parallel to this effort, we collected samples from two specialist sequestering species, monarch butterflies (*Danaus plexippus*) and large milkweed bugs (*Oncopeltus fasciatus*), reared on leaves and seeds, respectively, of both subspecies. Our work informs the state of PSM differences towards the end of the speciation process between these two milkweeds.

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## The Na,K-ATPase $\beta$ -subunit influences cardenolide distribution in the body fluids of a cardenolide-adapted insect

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The Na,K-ATPase is a vital transmembrane enzyme found in all animal cells. It is responsible for the maintenance of the membrane potential and the general functionality of cells. The minimal functional unit consists of one  $\alpha$  and one  $\beta$ -subunit. However, more than one paralog exists in different animal species. In our model organism, the large milkweed bug (*Oncopeltus fasciatus*), ancestral gene duplications have produced four  $\alpha$ -paralogs, which differ in activity and resistance to cardenolides. In addition, there are four  $\beta$ -subunits. Apart from chaperoning the associated  $\alpha$ -paralogs, the  $\beta$ -subunits have other functions that are not yet fully understood. The large milkweed bug is one of the few insect species that is resistant to cardenolides and even benefits from these plant toxins for its own defense. Cardenolides inhibit Na,K-ATPases by binding to a specific binding pocket located in the  $\alpha$ -subunit. The existence of four different  $\alpha$ -paralogs allows tissue-specific adaptation to toxin exposure in *O. fasciatus*. The NKA is also involved in the formation of septate junctions (SJs). At least in *Drosophila*,  $\alpha$  and  $\beta 2$  (Nrv2) are known members of the SJ protein core complex and contribute to the paracellular barrier function of the junctions. However, the role of the  $\beta$ -subunits in the context of cardenolide exposure has remained unknown until now. Here, we investigate the relationship between cardenolide exposure and SJ integrity, focusing on the specific roles of the four  $\beta$ -subunits. We found tissue-specific involvement of different  $\beta$ -subunits in the formation of SJs. Furthermore, knockdown of different  $\beta$ -subunits resulted in alterations of cardenolide distributions in hemolymph, defense fluid and feces. Preliminary results further suggest changes in cardenolide abundances in the generally well-protected nervous tissue after knockdown of  $\beta$ -subunits, thus supporting the idea that  $\beta$ -subunits play an important role in SJ formation.

**Keynote: Molecular mechanisms underlying agroecological pest control as practiced by smallholder farmers in sub-Saharan East Africa**

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The UPSCALE project aims to better understand mechanisms underlying yield improvements achieved through push-pull technology (PPT), an agroecological sustainable intensification strategy, and to lift barriers to its wider adoption. PPT uses a combination of intercrops and border crops to reduce pest load on a focal crop: most often maize; and is designed to meet needs of smallholder farmers in sub-Saharan East Africa. Volatiles emitted by the intercrop and border crop contribute to control insect pests, and root exudates from intercrops suppress parasitic *Striga* weeds. This mechanistic understanding is based on a small, but growing number of laboratory studies. We aimed to expand these studies to PPT as currently practiced by farmers and to test this laboratory-based understanding across the “translation gap” to in-field practice. We developed simple, field-robust sampling and extraction approaches allowing us to characterize samples from paired PPT and non-PPT fields run by farmers while preserving field chemical profiles. We characterized volatiles emitted from focal and companion crops, and the defensive metabolome of the focal crop under PPT or non-PPT cultivation. We also conducted bioassays to test the effectiveness of PPT intercrops against the fall armyworm, now a leading cause of maize yield loss across Africa. Our initial findings indicate that PPT fields have richer and more diverse plant volatile profiles, driven especially by the border crop. The relative importance of intercrop volatiles is less clear in comparison to other intercrop traits, which include nitrogen fixation and weed suppression, and acting as a non-host for herbivores of maize. We also find that, while maize crops across the study countries (Kenya, Rwanda, and Uganda) harbored substantial variation in their defense-related metabolomes, maize cultivated under PPT consistently accumulated higher levels of defensive benzoxazinoids. More detailed analyses of Kenyan fields indicated that this was accompanied by lower emission of herbivore-induced volatiles and less accumulation of stress-related flavonoid compounds in PPT-cultivated maize, which should, however, be interpreted in light of many country-specific differences. These results will be compared with data concomitantly collected by UPSCALE partners on arthropod populations inside and outside of fields, *Striga* weed abundance, and yield. We contribute reliable approaches for eco-metabolomics under logistically challenging field conditions that can yield new insights into chemical ecology supporting sustainable intensification.



## **Deciphering the defense strategies of wild mungbean against *Spodoptera litura***

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The tobacco caterpillar (*Spodoptera litura*) poses a significant threat to mungbean (*Vigna radiata*) crops, yet the plant's chemical defense mechanisms are not well understood. This study screened wild mungbean populations to identify resistant (R1) and susceptible (S1) accessions to *S. litura*. RNA-Seq analysis of R1 leaves subjected to various durations of larval feeding revealed the involvement of CaMK, MAPK, and jasmonic acid (JA) signaling pathways, which redirect resources from primary metabolism to the production of defense-related proteins and secondary metabolites. Comparing gene expression patterns between R1 and S1 plants exposed to larval feeding showed increased expression of CAMK, MAPK, and genes involved in terpene and flavonoid biosynthesis in R1 plants. Additionally, R1 plants exhibited higher JA levels, and metabolic profiling revealed elevated tryptophan and tyrosine levels, potentially leading to the accumulation of glucosylated kaempferols and the release of (E)- $\beta$ -ocimene. Interestingly, extracts from wild mungbean leaves caused growth retardation of caterpillars, shedding light on inherent defense mechanisms. To elucidate mungbean's indirect defense mechanisms, we will conduct olfactometer assays and compare them with volatile organic compound (VOC) profiles to identify key compounds modulating indirect defense responses. These findings enhance our understanding of wild mungbean interactions with herbivorous insects, offering potential implications for breeding programs to improve insect resistance.

## Prediction and validation of terpene synthase functions in *Tanacetum vulgare*

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Terpenoids are the largest and most diverse class of plant specialized metabolites, of which many are ecologically and economically important. A local population of *Tanacetum vulgare* is able to produce many different terpenoids with substantial standing variation raising the question of how this high level of chemodiversity evolves and is maintained. To understand the evolutionary patterns, we determined the products of a representative number of terpene synthase (TPS) candidates. We sequenced 24 transcriptomes and one genome of *T. vulgare*, assembled and annotated, and correlated the TPS abundance to metabolite levels to determine the candidates. Biochemical tests confirmed the predictions, demonstrating that transcript abundance can predict product abundance. Phylogenetic analyses suggest that in *T. vulgare* at least one type of TPS evolved de novo while others are already present in other Asteraceae. RNA-seq data suggests that different TPS at varying expression levels are present in one chemotype, suggesting they are not allelic but reside in different loci. We tested if alignments were able to determine the amino acid residues relevant for substrate specificity but were unable to pinpoint relevant amino acids. We also tested if homology modeling revealed obvious, large changes within the active site and were again unable to identify large changes. We thus conclude that subtle changes, likely away from the active site, alter the active site sufficiently to change the product. Currently, we use the genome of a *T. vulgare* individual with a chemotype dominated by beta-thujone to trace the evolutionary history of the TPS loci identified in biochemical analyses in this single chemotype. Our quantitative, population-level RNA-seq approach demonstrates its suitability for large-scale mining for new specialized metabolism enzymes for biotechnological applications based on chemodiverse plant populations.

## Natural rubber reduces herbivory and alters the microbiome below ground

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Laticifers are hypothesized to mediate both plant–herbivore and plant–microbe interactions. However, there is little evidence for this dual function. We investigated whether the major constituent of natural rubber, cis-1,4-polyisoprene, a phylogenetically widespread and economically important latex polymer, alters plant resistance and the root microbiome of the Russian dandelion (*Taraxacum koksaghyz*) under attack of a root herbivore, the larva of the May cockchafer (*Melolontha melolontha*). Rubber-depleted transgenic plants lost more shoot and root biomass upon herbivory than normal rubber content near-isogenic lines. *Melolontha melolontha* preferred to feed on artificial diet supplemented with rubber-depleted rather than normal rubber content latex. Likewise, adding purified cis-1,4-polyisoprene in ecologically relevant concentrations to diet deterred larval feeding and reduced larval weight gain. Metagenomics and metabarcoding revealed that abolishing biosynthesis of natural rubber alters the structure but not the diversity of the rhizosphere and root microbiota (ecto- and endophytes) and that these changes depended on *M. melolontha* damage. However, the assumption that rubber reduces microbial colonization or pathogen load is contradicted by four lines of evidence. Taken together, our data demonstrate that natural rubber biosynthesis reduces herbivory and alters the plant microbiota, which highlights the role of plant-specialized metabolites and secretory structures in shaping multitrophic interactions.

## Loss of olfaction through mutagenesis of the olfactory co-receptor Orco impairs foraging and natural enemy avoidance of *Pieris brassicae* caterpillars

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Herbivores are under strong selection pressure to forage efficiently and avoid natural enemies at the same time. In this study, we aimed to investigate the role which the odour guided behaviour of an insect herbivore plays in the tri-trophic interaction between the herbivore, its host plant and its natural enemies. We employed CRISPR/Cas9 to knock-out the odourant receptor co-receptor (Orco) in *Pieris brassicae* and compared the performance of knock-out (KO) and wild-type (WT) caterpillars in their interaction with the plant *Brassica oleracea* and the parasitoid *Cotesia glomerata*. We first confirmed the knock-out of Orco by staining odourant receptor neurons in antennae and the larval antennal centre in the caterpillar brain with an anti-body against the Orco protein. We found that a knock-out of Orco, not only resulted in a loss of staining in the caterpillar antenna, but also resulted in a significant decrease of the olfactory centre in the caterpillar brain. Electrophysiological measurements on the adult butterfly antenna further confirmed that KO animals lost most of their neuronal response to the tested chemicals. Having established that the KO mutants lost most of their olfactory responses, we investigated the chemical compounds potentially involved in natural enemy avoidance by analysing volatile chemicals from *P. brassicae* caterpillars, caterpillar spit and frass as well as *C. glomerata* female wasps. From this analysis, we selected 15 chemicals and tested the response of both KO- and WT-caterpillars to these volatiles. Wild-type caterpillars were attracted to cis-3-hexen-1-ol and trans-2-hexenal, while KO animals were insensitive to all chemicals. In addition, KO-caterpillars did not locate their host plants as efficiently as WT- caterpillars in a two-choice assay and grew less fast when feeding on *B. oleracea* plants. When challenged by parasitoid from which the stinger had been removed, making them unable to physically injure the caterpillars, the difference between KO- and WT-caterpillars increased further, indicating that KO-caterpillars were more prone to parasitoid attacks. The greater vulnerability ultimately resulted in a lower survival rate of the KO-caterpillars when confronted with unmanipulated parasitoids. Our results emphasise the role of chemical information for the foraging and natural enemy avoidance of insect herbivores and highlight the effects of the herbivores behaviour for multi-trophic interactions.

## Oviposition-induced plant volatiles prime undamaged rapeseed defences against upcoming herbivores.

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Plants exploit environmental cues about the risks of encountering insect herbivores. Responses to such cues often entail priming, sensitising prompt deployment of defences against herbivory. While most studies show that herbivore-induced plant volatiles (HIPVs) from herbivore-damaged plants prime undamaged receivers against incoming herbivores, priming by oviposition-induced plant volatiles (OIPVs) is under-explored. We studied whether egg-infested *Brassica napus* OIPVs prime defences in receiver undamaged *B. napus* when infested with *Pieris brassicae*. We collected and analysed the volatiles and measured *P. brassicae* larvae weight on OIPV-receiver and control plants as a proxy for defence. We show that exposure of undamaged plants to OIPVs promotes stronger plant defences against subsequent pest damage. Oviposition triggers the release of specific volatiles, i.e.  $\alpha$ -pinene, dimethyl-trisulfide, and limonene, potentially serving as herbivore early warning cues for receiver plants.

Furthermore, larvae weighed less when feeding on OIPV receivers for three and seven days than on controls. Exposure to OIPVs altered the volatile profiles of receiver plants. After three days of caterpillar feeding, OIPV-receiver plants released lower total volatiles, DMNT, nonaldehyde, and 4-hexenyl-acetate than controls. However, after seven days of herbivory, all plants released almost equal amounts of volatiles, regardless of treatment. We suggest a potential trade-off between direct and indirect defences, with primed plants investing metabolic resources initially towards direct and later enhancing indirect defences. These findings document that OIPVs mediate interactions between plants as herbivore early warning cues, contributing to understanding plant-plant communications and delivering insights into natural defence approaches for agroecological crop protection in Brassicaceae.

## Getting the big picture: Studying the ecological consequences of producing antimicrobial defences during root herbivory

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Plants are equipped with an extensive set of chemical and physical traits that together offer resistance against biotic stressors, including insect herbivores and pathogenic microorganisms. Many of these defence traits, including plant-specialized metabolites such as glucosinolates (GSLs), are pre-formed and present constitutively. Others are only synthesised after the recognition of a particular stress or are produced in increased concentrations. According to optimal defence theory (ODT), such inducible defences lower ecological and metabolic costs of resistance in the absence of attackers. They also fine-tune plant responses to specific organisms, thereby increasing their effectiveness. Nevertheless, not all herbivore-induced plant responses are as specific as predicted by ODT. In *Brassica* spp., insect herbivory triggers the accumulation of indole glucosinolates, tryptophan-derived secondary metabolites linked primarily to resistance against pathogenic microorganisms. In fact, a strong increase in indole glucosinolate biosynthesis may attenuate the production of aliphatic GSLs, compounds that actually confer resistance to chewing herbivores. Here, we assessed the ecological consequences of the increased production of indole glucosinolates and their derivatives in *Brassica rapa* upon root herbivory by the cabbage root fly (CRF) *Delia radicum*. In addition, we performed bioassays that included an additional root herbivore, the root-knot nematode *Meloidogyne incognita*, to study whether this plant pathogenic nematode may affect CFR performance. We combined induction experiments with targeted and untargeted metabolomics approaches as well as gene expression analyses to explore the underlying molecular mechanisms. We found that root-herbivory CFR larvae induce the accumulation of specific indole GSLs, as well as tryptophan-derived phytoalexins. Exposure to cyclobrassinin, a prominent phytoalexin in *B. rapa*, inhibits the growth of herbivory-associated bacteria, suggesting a benefit of producing antimicrobial compounds during root herbivory. However, prior infection by *M. incognita* facilitated CFR performance, potentially driven by trade-offs between indole and aliphatic GSL biosynthesis. Together, our results imply that the adaptive value of this conserved induced response should be studied in the appropriate ecological context, taking the presence of (opportunistic) pathogens into account.

## Molecular mechanisms of organ-specific steroidal glycoside diversity in *Solanum dulcamara* affecting insect-plant interactions: A bittersweet story

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Bittersweet nightshade (*Solanum dulcamara*) plants produce a large variety of steroidal glycosides (SGs). Minor chemical differences, for example the presence (unsaturated SG) or absence (saturated SGs) of a double bond in the steroidal moiety, are associated with differences in resistance to herbivores and pathogens [1]. Liquid Chromatography-quadrupole Time of Flight – Mass Spectrometry metabolomic analyses showed that both total leaf SG levels and SG diversity are associated with herbivore preference and leaf damage. We analysed the metabolomes of leaves and roots of selected chemotypes with saturated and unsaturated SG profiles in their leaves. In addition, we assessed the expression levels of a homolog of the GLYCOALKALOID METABOLISM25 (GAME25) gene involved in the structural diversification of steroidal alkaloids in cultivated Solanaceae. In tomato, GAME25 is coding for a short-chain dehydrogenase is responsible for the reduction of the C-5,6 double bond in dehydrotomatidine, which results in the formation of tomatidine [2]. Our results showed that differences in leaf SG chemodiversity were associated with the expression. In contrast to leaves, roots of the same saturated and unsaturated individuals were more similar in their SG profiles and GAME25 expression levels. This implies that leaf and root SG chemodiversity in *S. dulcamara* are under differential selection pressures by aboveground and belowground interactions with herbivores and pathogens.

1.Calf, O.W., et al., Glycoalkaloid composition explains variation in slug resistance in *Solanum dulcamara*. *Oecologia*, 2018. 187(2): p. 495-506.

2.Sonawane, P.D., et al., Short-chain dehydrogenase/reductase governs steroidal specialized metabolites structural diversity and toxicity in the genus *Solanum*. *Proceedings of the National Academy of Sciences*, 2018. 115(23): p. E5419-E5428

## **A sweet starter? *Solanum dulcamara*'s wound nectar - An inheritable and polymorphic trait**

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Nectar is a common plant trait to attract beneficial animals like pollinators. Extrafloral nectaries attract predacious animals, commonly ants, which defend plants against herbivores. *Solanum dulcamara* secretes a new form of extrafloral nectar without bearing any nectaries: It is directly released at herbivore-inflicted wounds and this wound nectar (WN) also attracts ants for plant defence[1]. Ant-attracting wound saps have been recently found in some plant species[2]. A hypothesis that could explain the frequent losses and gains of nectaries present throughout the plant kingdom[3], is that WN secretion might represent an early step in nectar evolution. Hence, we aim to investigate the mechanisms underlying *S. dulcamara*'s WN secretion and examine the phenotypic variability of this trait and its relation to environmental conditions. We developed approaches to discover and functionally test genes that may be associated with WN secretion and characterised the phenology of *S. dulcamara*'s WN secretion, which is enhanced under drought conditions and variable between geographically distinct accessions. We determined WN secretion as an inheritable trait that is segregating in a population of plants originating from America, which we want to use for genetic correlations to identify genes determining the trait. Using CRISPR-Cas9-mediated mutation, we investigated role of candidate genes within sugar transport/metabolism and plant signalling. We discovered that loss of wound-induced JA-biosynthesis results in higher WN production despite JA-signalling activates WN secretion. As JA-signalling is required for the majority of the plant's wound response but not for WN production, this mutant may help us to identify genes involved in the molecular mechanisms underlying WN secretion.

[1] Lortzing T. et al. (2016) Nature Plants. 2: 16056

[2] Staab M. et al. (2017) Am. Nat. 190: 442-450

[3] Weber M.G. and Keeler K.H. (2013) Ann. Bot. 111: 1251–1261



**Keynote: Convergence and divergence in the diversity of insects feeding on a toxic plant**

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I will propose a framework in organismal biology for studying strategies (or syndromes of traits) associated with specialization on a toxic resource. Using milkweed as a focal plant, fed upon by monarch butterflies and a taxonomically diverse set of other insect herbivores, I will address how we should we interpret strong phenotypic convergence. Using crispr and comparative biology we'll work towards some answers. Perhaps convergence and divergence both contribute to organismal diversity. I hypothesize that multispecies coevolution results in a diversity of plant defenses which in turn interact in complex ways with insect communities. This slightly modified view of classic coevolution is predicated on commonalities and differences between species as a generator of biodiversity.

## **Vertical stratification of leaf physical traits exerts bottom-up pressures on insect herbivory in a sugar maple temperate forest**

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Do vertical gradients in temperate forest structure insect herbivore communities? We tested the hypothesis that the increase in light intensity from understory to forest canopy level drives differences in leaf physical traits and budburst phenology that impact insect herbivores and thus play a role in structuring both herbivore communities and the leaf damages they cause. Twelve sugar maple (*Acer saccharum*) sites were monitored in southern Quebec, examining insect herbivore patterns from understory to the shaded and sun canopy over the summers of 2020, 2021, and 2022. Additionally, we recorded leaf physical traits, temperature, humidity, and sun exposure. Our findings revealed that leaf thickness increased along the vertical gradient in 2021, making leaves less favorable to herbivores in canopy level. Accordingly, we recorded a consistent decrease in insect herbivory damage rates from the understory to the shaded canopy and sun canopy in 2020 and 2021, driven by leaf cutters, skeletonizers, stipplers, and leaf miners. These results support our hypothesis that variation in plant physical traits due to sun exposure contributes to the vertical stratification of insect damage. In 2022, the gradient of insect herbivore abundance corroborated the observed damage trends from the previous years. Moreover, we calculated an average annual herbivory rate of 9.1% of the leaf surface in our study site, suggesting limited evidence supporting a significant contribution of background herbivory to the decline of sugar maple forests. Overall, our study highlights the importance of vertical gradients in structuring insect herbivore communities and emphasizes the role of leaf traits in mediating these interactions.

## Plant defense under arctic light conditions: Plants vs pests

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The anticipated effects of global warming extend to altering the growth conditions for plants and crops in regions situated at high latitudes, particularly those beyond 60° N, such as the Arctic. Consequently, there will likely be shifts in the natural composition of plant and pest communities, as herbivorous arthropods expand into these territories. Such expansions may instigate novel interactions between species that previously had minimal overlap, introducing fresh challenges in combatting herbivore attacks. Interestingly, plant species thriving in high latitude environments historically face reduced levels of herbivory in comparison to those found at lower latitudes. Our hypothesis is that this phenomenon stems from a gradient of inherent chemical defense mechanisms towards the Northern regions (1). We further hypothesize that this heightened defense is facilitated by elevated levels of the defense-associated phytohormones such as jasmonate. Given its dependence on light for biosynthesis, the extended daylight hours characteristic of Arctic summers may foster the accumulation of jasmonate and consequently trigger downstream physiological responses. To test these hypotheses, we conducted LC-MS/MS based metabolic profiling, gene expression analysis employing RNAseq, and classical feeding assays comparing Bilberry plants (*Vaccinium myrtillus*) that naturally grow in temperate and arctic regions. Preliminary metabolic studies have revealed significant differences in metabolic composition and expression intensity between plants of the two regions. The variability in metabolic expression, including jasmonates, throughout the summer season is notably lower in the arctic region, indicating a steady metabolic rate likely attributed to the consistent light regime during the summer. Among the metabolites overexpressed in the Arctic compared to the temperate region, approximately 70 of them exhibited sustained overexpression throughout the summer months, with the majority involved in the shikimate-phenylpropanoid pathways, recognized for their role in plant defense against insect herbivory.

(1) Mithöfer A, Riemann M, Faehn CA, Mrazova A, Jaakola L (2022): Plant defense under Arctic light conditions: Can plants withstand invading pests? *Front Plant Sci* 13, 1051107

## Quantifying functional diversity-index via UV-Vis spectral identity: A novel route to compound dissimilarity matrix

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Understanding ecological and evolutionary significance of plant chemodiversity remains a central pursuit in chemical ecology. Chemodiversity is often assumed to be driven by variability of environmental factors such as herbivory, pollination, and abiotic factors over evolutionary time scales. Different approaches to quantify chemodiversity in ecologically and evolutionary meaningful ways have been suggested. Yet, most measures of phytochemical diversity do not account for structural properties of the compounds measured. As structural properties relate to the compounds' function and their biosynthetic (in)dependence, a method considering structural dissimilarity in addition to richness and evenness in compound abundance has been recently proposed<sup>1</sup>. We aimed to apply this framework of the so-called functional diversity-index (FD-i) to a metabolic screening of *Solanum dulcamara* leaf chemistry. However, an important limitation for quantifying chemodiversity using FD-i is its confinement to identified compounds. Approaches to quantify compound dissimilarity are heavily based on structural information (e.g. NPClassifier, PubChem Fingerprint, fMCS). Therefore, these approaches require characterisation of the compounds either by NMR- or mass spectra and thus advanced analytical instrumentation. Yet, parallel identification and quantification of compounds in crude plant extracts is often compromised by trade-offs between scanning breadth for structural identification and high sensitivity for reliable quantification. Additionally, pre-processing data for identifying and quantifying numerous compounds from chemically complex crude plant extracts is labour intense. As a cost-effective and simple alternative approach, we tested a novel method to assess FD-i using pair-wise (dis-)similarities of UV-Vis spectra from HPLC-DAD (High-performance liquid chromatography coupled with diode-array detector) analyses of phytochemical mixtures. We further tested this approach on a data set, for which more than 100 individuals growing in nine natural *S. dulcamara* populations were sampled in the field and evaluated FD-i using the CHEMODIV R-package [1]. Our approach to calculate compound dissimilarity opens new opportunities to expand the usage of FD-i and thus test for its relevance in eco-evolutionary contexts, bridging the gap between necessity to have complete structural details and rapid screening of phytochemicals.

[1] Petren et al. (2023) *New Phytologist* 237: 2478–2492

## **Ecological conflicts of floral defenses in the high Andean shrub *Bejaria resinosa* (Ericaceae)**

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The stickiness of vegetative leaf tissue represents a relatively common and effective plant defense against herbivores, which has evolved independently in multiple plant families. It is markedly rarer as a trait of floral tissues, specifically petals, likely because it may compromise interactions with mutualists, such as pollinators. However, as a strong defense trait floral stickiness may also represent a floral filter not only reducing florivory but also optimizing interactions with the community of potential pollinators. The High-Andean shrub *Bejaria resinosa* (Ericaceae) is dimorphic for petal stickiness and thus represents a convenient model system to test hypotheses of conflicting ecological effects associated with chemical defenses. Here we test the hypothesis that floral stickiness functions as an anti-herbivore defense and assess the associated ecological costs that come with potentially compromised interactions with pollinators. We demonstrate that stickiness functions as a defense against folivores but the effects on seed set are strongly dependent on the community context within which stickiness is expressed. This context-dependency of chemical defense functionality likely arises from the differential availability of primary pollinators and potential trade-offs between chemical defense and pollinator attraction.

## Understanding the effects of biogeography and domestication on a facultative ant-plant mutualism in an ancient alien species

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The Biotic Interactions hypothesis predicts that species interactions intensify from the poles to the tropics. We investigated the interplay of latitude and domestication in shaping the ecology of the facultative mutualistic interaction between castor oil plants (*Ricinus communis*) and ants. Castor has extrafloral nectaries that attract ants, which likely protect the plant from damage by herbivores. Castor was domesticated in Africa, introduced to India over 2000 years ago, and it is now naturalized and extensively cultivated to extract oil, making India the world's largest producer of castor beans. Agricultural varieties may have evolved weaker mutualisms as they thrive in managed environments. Little is known about gene flow between wild and cultivated populations.

In 2022, we sampled mutualism in naturalized castor populations across a 20-degree latitudinal range in India. Then, in 2023, we planted seeds from these populations and prominent agricultural varieties in a common garden in southern India, where the highest ant species richness was observed in 2022. We predicted that plants from southern, more tropical habitats would invest more in mutualism, interact with more diverse ants, and have higher fitness than northern varieties. We also predicted that agricultural varieties would invest less in mutualism and attract fewer ants.

Herbivory was higher and investment in mutualism was lower in higher latitudes in naturalized populations, but the trends flipped in the common garden, presenting conflicting patterns. In the common garden, agricultural varieties produced more nectaries but attracted fewer ants. There was no evidence of a home site advantage in plants from southern sites. Our study presents a test of the Biotic Interactions hypothesis from a large latitudinal gradient in India for both mutualism and herbivory. Domestication reshapes natural species interactions, and understanding these phenomena will enable us to leverage the natural histories of agriculturally important species in developing sustainable agricultural practices.

## Immature leaves are the dominant volatile sensing organs of maize

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Plants perceive herbivory induced volatiles and respond to them by upregulating their defenses. So far, the organs responsible for volatile perception remain poorly described. Here, we show that responsiveness to the herbivory induced green leaf volatile (Z)-3-hexenyl acetate (HAC) in terms of volatile emission, transcriptional regulation and jasmonate defense hormone activation is largely constrained to younger maize leaves. Older leaves are much less sensitive to HAC. In a given leaf, responsiveness to HAC is high at immature developmental stages and drops off rapidly during maturation. Responsiveness to the non-volatile elicitor ZmPep3 shows an opposite pattern, demonstrating that this form of hyposmia (i.e. decreased sense of smell) is not due to a general defect in jasmonate defense signaling in mature leaves. Neither stomatal conductance nor leaf cuticle composition explain the unresponsiveness of older leaves to HAC, suggesting perception mechanisms upstream of jasmonate signaling as driving factors. Finally, we show that hyposmia in older leaves is not restricted to HAC, and extends to the full blend of herbivory induced volatiles. In conclusion, our work identifies immature maize leaves as dominant stress volatile sensing organs. The tight spatiotemporal control of volatile perception may facilitate within-plant defense signaling to protect young leaves, and may allow plants with complex architectures to explore the dynamic odor landscapes at the outer periphery of their shoots.

## Plant induced defenses promote transgenerational effects including intraclutch cannibalism in Colorado potato beetle

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Animals need to cope with an ever-changing environment. One mechanism by which parents can increase their progeny's fitness is through transgenerational effects, the transmission of traits or responses from parents to offspring influenced by environmental factors encountered by the parental generation. Certain traits, such as short generation times and limited dispersal, foster a strong autocorrelation of habitat conditions across generations, thereby increasing parents' ability to predict their offspring's environmental conditions, enhancing these effects. Parental nutrition is a significant driver of transgenerational effects, impacting offspring traits such as hatching success, growth, development, and immunity. Therefore, plant quality alterations due to the induction of anti-herbivory defenses can affect both the herbivorous insects feeding on the plant and their offspring.

Here, we explored whether temporal changes in plant quality due to defense induction trigger adaptive transgenerational effects in Colorado potato beetles (*Leptinotarsa decemlineata* Say, CPB). Initially, we assessed the direct impact of induced plant defenses on adult CPB reproductive success and whether it promotes parental provisioning in the form of intraclutch cannibalism. Subsequently, we carried out a reciprocal experiment to explore whether cross-generational environmental match increases offspring fitness and the effect of cannibalism on CPB larvae feeding and development.

We found that induced defenses, known for lowering plant quality as a resource for insect herbivores, significantly reduce adult CPB oviposition and impede larval growth. Notably, induced plant defenses also facilitated transgenerational effects. Offspring of parents who consumed defended plants exhibited an increased rate of intraclutch cannibalism, which in turn accelerated larval growth, especially when reared on induced plants. Furthermore, we found that larvae exhibit improved performance when reared on the same type of plant their parents encountered, indicating the presence of additional non-genetic transgenerational effects beyond the enhancement of cannibalism.

Focusing on plant-induced defenses, our study broadens the spectrum of environmental factors influencing transgenerational effects. Additionally, the transmission of phenotypic traits across generations is added to a diverse array of strategies developed by insect herbivores in their enduring coevolutionary arms race with plants.



## **Keynote: Aphids as sensors: Exogenous application of allelochemicals and aphid probing behavior**

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Aphids (Hemiptera: Aphididae) are herbivores with specialized piercing-sucking mouthparts. Aphid probing behavior is defined as a set of various activities associated with the mouthparts' stylets penetration in plant tissues. During probing, aphids collect samples of sap, first - from peripheral plant tissues and, second – from sieve elements, the basic aphid food source. At each phase of probing, allelochemicals detected in these sap samples may alter aphid foraging behavior. Depending on whether an allelochemical is a phagostimulant or a phagodeterrent, aphids may continue probing and/or feeding or withdraw the stylets. Therefore, the parameters describing aphid behavior during probing are good indicators of plant suitability or the interference in probing by chemical or physical factors characteristically present in individual plant tissues. At present, aphid control depends mainly on the use of neurotoxic insecticides. Due to the repeated applications, many aphid species have developed resistance to several aphicides. The use of targeted chemicals that would repel aphids or deter their probing and feeding is one of the most promising approaches. Therefore, the understanding and modification of aphid behavior during the pre-ingestive and ingestive phases of probing are crucial in designing alternative methods of aphid control.

Following the biopesticide-related approach to aphid control and reduction of virus transmission, we present the results of our multi-year research on aphid probing behavior-modifying activity of several natural and structurally modified allelochemicals. We show that using aphids as sensors it is possible to demonstrate indirectly that the exogenously applied xenobiotics may penetrate the plant cuticle and epidermis and pass into deeper tissue layers. We also show that the transcuticular application of certain allelochemicals may cause disturbances in plant recognition and acceptance, which may finally reduce aphid infestation. In our experiments we applied the electrical penetration graph technique known also as electropenetography or EPG, which visualizes the movements of aphid mouthparts' stylets in individual plant tissues.

## **Plant part-specific niche construction by the specialist aphid *Sitobion avenae* on wheat**

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Aphids are phloem sap-feeding herbivores and often tremendous pests on plants including agricultural crops. These herbivores may modify plant quality, for example by affecting the concentrations of nutrients such as amino acids or defensive compounds. Aphid-induced changes in their host plants may in turn affect the performance of the colonising aphids as well as their preference behaviour.

In this study, we investigated the effects of a previous infestation of wheat leaves or ears with the English grain aphid (*Sitobion avenae*) on conspecific aphids of another or of the same clonal lineage. Moreover, the chemical composition of phloem exudates of the corresponding plant parts was assessed after aphid infestation, applying amino acid profiling as well as metabolic fingerprinting, with the latter approach covering mainly specialised metabolites.

For the leaves, previous infestation by aphids led to slight and transient positive effects on the colony sizes of conspecifics. However, aphids did not show a preference for any of the differently treated leaves. These findings were similar for conspecifics of another as well as for those of the same clonal lineage. In the phloem exudates of the leaves, only minor metabolic changes after aphid infestation were found. On the contrary, in wheat ears a previous infestation had strong, long-lasting positive effects on aphid colony sizes, accompanied by a preference for the previously infested ears, both for another as well as for the same clonal lineage. The phloem exudates of the aphid-infested ears showed higher proportions of asparagine and different relative concentrations of several putative specialised metabolites.

Taken together, these results indicate plant part-specific niche construction by *S. avenae*, with positive effects on aphid fitness as well as a preference for the constructed niches. The effects are probably at least partly due to aphid-induced changes in the phloem sap of the plants. The higher proportion of asparagine in phloem exudates of ears after aphid infestation may indicate an accelerated nitrogen mobilisation to support grain filling, which may facilitate the aphids. This study deepens our understanding of aphid-plant relationships and highlights the dynamic nature of such interactions. Niche realisation processes such as niche construction and niche choice probably largely contribute to the success of aphids when interacting with their host plants.

## A comparative proteomic and metabolomic study of phloem sap from sweet orange and lemon trees infested by *Trioza erytreae*

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European citriculture is on high alert due to the threat of Huanglongbing (HLB), a highly harmful bacterial disease caused by *Candidatus Liberibacter*. The psyllid vector *Trioza erytreae* recently incurred in the Iberian Peninsula, albeit not carrying the agent of HLB. The psyllid feeds on the hosts' phloem sap and nymphs develop on leaves. Sweet orange (*Citrus × sinensis*) (SwO) is a common host of *T. erytreae*, whilst lemon (*Citrus × limon*) is the preferred host. To unveil the response of both citrus trees to *T. erytreae*, the phloem sap of infested and not infested hosts was compared using a multiomics approach. Citrus hosts were infested with five male and five female *T. erytreae* adults per tree, or not infested. Three times more nymphs developed in lemon trees. Phloem sap was extracted from the leaves and analysed by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) for metabolic profile, with 39 target metabolites. Infested trees yielded 10 and 14 differentially abundant metabolites (DAM) ( $F < 0.05$ ) for SwO and lemon trees respectively. In SwO trees upregulated DAMs included succinate, malate and aspartate, suggesting a broad metabolic adjustment, related to the citrate cycle (TCA cycle) and carbon fixation in photosynthetic organisms' pathways. The defence related metabolites pipercolate and jasmonate were also upregulated in SwO. In contrast, phenylalanine, p-coumarate was upregulated and ferulate was downregulated in lemon trees indicating enrichment of the stress coping phenylpropanoid biosynthesis pathway. Proteomic analysis by nanoscale liquid chromatography coupled to MS/MS (nanoLC-MS/MS) yielded 1265 and 48 differentially abundant proteins (DAP) ( $F < 0.05$ ) in SwO and lemon trees, respectively. In SwO trees the DAPs enriched 86 pathways revealed an overlap with the metabolomic results, in relation to the jasmonate related alpha-linolenic acid metabolism pathway. Only 10 enriched pathways were represented by the DAPs of lemon trees, only one related to plant defence. The present results provide insights into the phloem sap molecular adjustment to *T. erytreae* infestation by the two citrus hosts. While in SwO trees a defence related response was observed, in lemon trees stress tolerance to the psyllid was noticed, which possibly interfered in *T. erytreae* development in lemon.

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## Comparative osmoregulation strategies and host plant adaptation in *Bemisia tabaci*

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Phloem sap is a nutrient-rich food source and the exclusive source of nutrients for most Hemipterans. These insects must overcome the high osmotic potential to exploit phloem sap, primarily due to its high sucrose concentration. The cryptic, polyphagous, phloem-feeding whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) can feed on a wide range of host plants. However, the role of osmoregulation strategies in plant adaptation remains poorly understood. In this study, we examined the honeydew secretions of two *B. tabaci* species: Sub-Saharan Africa (SSA), which infests cassava, and Middle East-Asia Minor 1 (MEAM1), which does not. Our goal was to elucidate differences in osmoregulation strategies between these species and their relationship to host plant utilization. After feeding on a common host plant (e.g., eggplant, kale), both species managed osmotic pressure similarly in the adult stage, with no significant differences in honeydew sugar profiles. Both species primarily produced sucrose isomers, such as trehalulose, to cope with osmotic pressure. However, when we tested the nymphs' sugar profiles, we found that SSA nymphs excreted sucrose more efficiently and had a higher ratio of oligosaccharides to isomers than MEAM1 nymphs. Furthermore, we found that plant secondary metabolites significantly impact honeydew sugar profiles. For instance, adding linamarin (a cyanoglucoside found in cassava plants) to artificial diets increased the production of oligosaccharides in adult honeydew. We suggest that SSA's adaptation to cassava plants may be attributed to efficient sucrose excretion and a high ratio of oligosaccharides to isomers. In addition, transcriptomic analysis revealed differential expression of osmoregulation genes in the two species, including glycoside hydrolase 13, sugar transporters, and aquaporins. These results suggest that SSA's ability to produce oligosaccharides confers a selective advantage when adapting to cassava.

## Identifying and characterizing members of the glycoside hydrolase family 13 with sucrose isomerase activities in *Bemisia tabaci*

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A phloem sap-based diet requires unique enzymatic skills for regulating the osmotic pressure differences in the gut lumen between the sugars-rich ingested sap and the body fluids. In the phloem-feeding whitefly *B. tabaci*, members of the GH13 family are likely to play a critical role in mitigating this threat. This is due to their high copy number in the genome, expression patterns, and enzymatic activities involved in sucrose metabolism: hydrolysis followed by oligomerization or isomerization which allows the reduction of the gut fluids osmolarity. While GH13 enzymes with sucrose hydrolysis or sucrose hydrolysis followed by transglucosidase activities have been previously identified and characterized in *B. tabaci* and other phloem feeders, the identity of the family members acting as sucrose isomerases remains unknown, although the dominant sugar found in the honeydew of *B. tabaci* is the sucrose isomer trehalulose, and it is known that bacterial sucrose isomerases have been classified under the GH13 family. In this study, we focused on four putative sucrose isomerase coding genes of *B. tabaci* belonging to the GH13 family, which were selected based on their expression patterns and conserved sequence motifs. We used artificial diets of sucrose and dsRNA to knock-down each of the target genes and analyzed the sugar composition of the secreted honeydew. We found that in all cases, the gene silencing resulted in a significant reduction in the fractions of trehalulose accompanied by an increase in the fractions of the trisaccharide melezitose, suggesting that the insects attempt to compensate for the inability to isomerize sucrose by synthesizing oligosaccharides – a well-known osmoregulation strategy of aphids and other phloem feeders. In addition, we found that the downregulation of the target genes also resulted in a significant increase in the mortality rates of the silenced insects and a significant delay in the development of their progeny, which indicates that the shift in the sucrose metabolism strategy has a dramatic effect on the fitness of *B. tabaci*. These findings demonstrate the importance of the osmoregulation-by-isomerization strategy in *B. tabaci*. Moreover, the genes characterized here can be used for developing RNAi-based pest management technologies. In addition, we are now a few steps closer to finding the *B. tabaci* trehalulose synthase enzyme/s, which could allow the efficient industrial production of trehalulose, a healthy sucrose substitute.

## Mealybug honeydew: How does its composition change under elevated CO<sub>2</sub> and water limitation?

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Honeydew produced by phloem sap-feeding insects is an important source of energy and nutrients for parasitoids and other natural enemies of crop pests. In perennial agroecosystems like vineyards, natural enemy populations can establish over years and contribute considerably to biological pest control. Climate change factors can affect host plant quality and, thus, trigger plant- and insect host-mediated bottom-up effects on honeydew-feeders. Mealybugs like *Planococcus ficus* (Hemiptera: Pseudococcidae) are an increasingly important pest feeding on grapevine (*Vitis vinifera*), but their honeydew is understudied compared to aphid honeydew.

We conducted a two factorial experiment to investigate the composition of *P. ficus* honeydew and its potential changes under climate change. We collected honeydew from mealybugs feeding on grapevine cultivated in climate chambers under ambient or elevated CO<sub>2</sub> concentration (400 and 750 ppm, respectively) with ample or limited water supply (water potential: -0.27 to -2.04 MPa). Honeydew metabolites were analyzed via gas chromatography coupled to mass spectrometry.

Several primary metabolites could be identified in the honeydew of *P. ficus*, including different sugars, free amino acids, and further organic acids. A non-metric multidimensional scaling analysis revealed a stronger impact of water limitation than of CO<sub>2</sub> concentration on the chemical composition of the honeydew, with the chemical profiles of the honeydew showing a gradient along with the water potential of the host plants. Moreover, the concentrations of some individual compounds were significantly affected by the CO<sub>2</sub> concentration and/or by water limitation of the host plant.

The chemical changes in the honeydew of the mealybug induced by elevated CO<sub>2</sub> and/ or water limitation of the host plant are probably at least partly due to modulations of the chemical composition of the phloem sap of their host plants by the climate change-related factors. Physiological adjustments by the mealybug may also play a role. Since honeydew-derived sugars and other metabolites can be of paramount importance for parasitoids of honeydew-producing insects but vary in their individual nutritional quality, fitness of the *P. ficus* natural enemy *Anagyrus vladimiri* (Hymenoptera: Encyrtidae) will be investigated as a next step to assess the potential impact of climate change on future natural enemy population dynamics in vineyards.

## Rhizobacteria induced transcriptomic changes to aphids on cereal crop barley

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Soil microbes, including fungal and bacteria symbioses modulate plant-insect herbivore interactions. Still, it remains unclear how these shapes the overall plant response and the mechanisms involved. We investigated how rhizobacteria modulate plant responses to aboveground insect herbivores. We performed bioassays with barley (*Hordeum vulgare*) plants to investigate the effect of two rhizobacteria species *Acidovorax radialis* and *Bacillus subtilis* on plant responses upon leaf herbivory by the aphid *Sitobion avenae*. By performing RNAseq analyses, we examined the impact of individual inoculations of the rhizobacteria on the transcriptomic changes triggered by the aphid, over three time points. Our transcriptomic analyses revealed that rhizobacteria inoculation in the absence of aphids mostly suppressed defence related genes. Aphid feeding on uninoculated plants triggered expression of defence related genes, whereas on rhizobacteria-inoculated plants we observed differential regulation of genes related to chemical defence and nutritional responses. These responses varied over time. with defence-related responses induced during initial aphid infestation (24 hours), and after sustained aphid population growth (21 days) timepoints, while nutritional responses were strongly induced during aphid colony establishment (7 days). These elucidated shifts in barley transcriptomic changes hold promise for exploitation towards sustainable crop protection and pest management in agriculture.

## Endosymbionts modulate virus effects on aphid-plant interactions

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Aphid-transmitted viruses frequently alter traits of host plants and aphid vectors in ways that enhance virus transmission [1]. Meanwhile, aphid interactions with host plants can also be modulated by the presence of aphid endosymbionts, whose fitness interests may diverge from those of viruses, but few studies to date have examined such interactions [2]. Moreover, the interface between disease transmission and microbial ecology is only beginning to be explored.

We investigated how different aphid endosymbionts influenced the effects of pea enation mosaic virus (PEMV) on interactions between pea aphids (*Acyrtosiphon pisum*) and fava beans (*Vicia faba* L.). In a series of performance, behavioral and metabolomics assays, we assessed how effects of virus infection differed for clonal aphid lines harboring only the obligate or each of four facultative endosymbionts [3].

Our results reveal striking interactions between infection status and the presence of specific endosymbiont strains [3]. Two particular aphid lines, harboring *Regiella insecticola* Ri or *Hamiltonella defensa*, exhibited strong interactions with the virus, typically in ways that favor transmission. For example, aphids harboring these endosymbionts had higher population growth or biomass on virus-infected plants. Virus infection also affected the host-plant preferences of these aphids, with initial preferences of virus-free aphids for infected plants being reversed to uninfected plants once they were vectoring the pathogen. Additional analyses of metabolites revealed that aphids harboring these endobacteria had higher levels of critical amino acids, sugars, and neuromodulator molecules when feeding on virus-infected plants, which indicates some of the mechanisms involved in altered aphid traits. Finally, a transmission assay revealed highest rates of PEMV transmission by aphids harboring *H. defensa* (but not *R. insecticola* strain Ri).

Altogether, these results demonstrate that the presence of different facultative aphid endosymbionts can modulate virus effects on aphid-plant interactions, with potential implications for disease transmission. More generally, these findings contribute to a growing body of work documenting the important role of microbial symbionts in shaping interspecific ecological interactions.

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## Soil type has drastic influence on corn leaf aphid performance

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The intricate relationship of soils, plants, and microorganisms underpins ecosystem vitality, influencing both plant growth and resilience to pest insects. Plant-soil feedback can influence plant chemistry and plant-herbivore interactions. Cover cropping, a common agricultural practice, leaves a legacy effect that alters soil carbon (C) to nitrogen (N) ratio, impacts herbivory resistance in maize, and can influence soil microbiomes. Despite its importance, the study of the soil legacy effects of cover crops on corn leaf aphid (CLA), *Rhopalosiphum maidis* is limited. To address the gap, we conducted experiments with CLA on a maize under greenhouse conditions using soils from three cover crop soils: legume-Austrian winter pea (*Pisum sativum* L.), non-legume broadleaf-forage radish (*Raphanus sativus* L.), grass-triticale (x *Triticosecale*), and potting mix as a control. We hypothesized that cover crops impart soil legacy effect conferring subsequent crop plant defense, thereby influencing defense against aphids. Eight treatments using four above-mentioned soil types with autoclaved and non-autoclaved soil were used to tease apart the role of soil microbes from soil nutrients in aphid responses to different cover crop soils. Ten adult aphids were introduced on the corn plant and the population was monitored. Enzymatic assays and plant volatile organic compound collection from each plant were performed to understand the effect of cover crops on corn direct and indirect defenses against aphids. Our findings suggest that irrespective of sterilization, field soil, regardless of which cover crop was present, reduced CLA populations. We will discuss our results highlighting the role of soil traits that may be causing this interaction.

## **Keynote: Down-to-earth defence: How grasses use silicon against insect herbivores in a multitude of ways**

Scott Johnson

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'Each blade of grass has its spot on earth whence it draws its life, its strength' wrote the novelist Joseph Conrad [1]. This description was more accurate than he may have realised, grasses literally acquire strength and resilience from the soil in the form of silicon. In fact, silicon is arguably the most fundamental anti-herbivore defence in the Poaceae, of which just three species provide 42% of human calories globally. Silicification of plant tissues makes them physically stronger, more difficult to manipulate and reduces the nutritional quality of foliage. But beyond this, silicon accumulation integrates with the wider defensive response of the plant and is regulated by the jasmonic acid pathway [2]. Our recent work has shown that silicon is a rapidly induced and persistent anti-herbivore defence which can be quickly activated in plants previously deficient in silicon. Indirect defences, which include natural enemies of insect herbivores, are reported to be boosted by silicon either via plant VOC signalling [3] or compromising the immune system of insect herbivores to their antagonists [4, 5]. Eating silicon-rich plant tissues can even rob an insect herbivore of cryptic colouration, leaving it susceptible to predators. Silicon defences are so effective, in some instances, that plants use mutualists (e.g. mycorrhizal fungi) to acquire more silicon defences. There are significant gaps in our understanding of these silicon-based interactions, but I argue that a better understanding of these ecological interactions might provide a route to more effective pest management in some agroecosystems.

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## Elemental defense: Harnessing zinc for enhanced rice protection against *Cnaphalocrocis medinalis*

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Rice (*Oryza sativa* L.), an important staple crop in Asia, faces many environmental stresses during cultivation, such as diseases and insect attacks, which severely reduce its yield. Previous studies have shown that increased copper treatment can enhance rice resistance to *Cnaphalocrocis medinalis*, but the effects of other metals, such as zinc (Zn), remain unclear. In this study, rice plants were treated with different concentrations of Zn, including a control, Low Zn, and High Zn, to explore the physiological and molecular mechanisms underlying their resistance to *Cnaphalocrocis medinalis*. As a result, the Zn-treated rice showed delayed growth and development of larvae, increased mortality, decreased pupae weight, and an increased number of abnormal adult moths. Through the whole-genome transcript sequencing (RNA-seq) analysis, rice chitinase genes were induced by Zn treatments and, therefore, changed the contents of chitinase, which may affect the development of larvae by affecting intestinal stability and food digestion and absorption. In addition, Zn treatments enhanced the expression of diterpenoid biosynthesis genes, and then increased the accumulation of diterpenoid, which may be related to rice's disease and insect resistance. In summary, the accumulation of Zn in rice directly caused metal poisoning to the larvae of *Cnaphalocrocis medinalis* and indirectly induced enzyme reactions or secondary metabolites, thereby increasing its defense against herbivores. In terms of agricultural application, we confirm the role of Zn in the application of herbivory insect defense.

## Inorganic versus organic defences of plants against herbivorous insects: Insights from hyperaccumulators

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Globally, more than 700 plant species are able to accumulate extremely high concentrations of metal(loid)s in above-ground tissues, termed hyperaccumulators [1]. *Raison d'être* for hyperaccumulation is evidenced by the elemental defence hypothesis (EDH), which postulates that metal(loid) hyperaccumulation acting as inorganic defences is potent against a range of antagonists, including herbivorous insects [2]. Similar to many other non-accumulating species, hyperaccumulators also synthesise a plethora of specialised metabolites acting as organic defences and they can differ qualitatively and quantitatively within species or even within an individual, known as chemodiversity [3]. Chemodiversity as a fascinating dimension of hyperaccumulators, however, is still neglected in the EDH. Here, we aim to showcase our recent studies on chemodiversity aspects in hyperaccumulators. We examined: (1) the status and position of chemodiversity in the EDH, (2) the relationships between inorganic versus organic defences and (3) how such distinct types of defences influence the performance of herbivorous insects. Combining literature and experimental studies, we found that: (1) inorganic and organic defences in hyperaccumulators were highly diverse, but only a handful of studies considered chemodiversity aspects when testing the EDH, (2) such defences could be metabolically interwoven as shown particularly in Brassicales hyperaccumulators, but potential trade-offs or joint effects between them were less clear-cut across studies, and finally, (3) an emerging model of Brassicales hyperaccumulator *Arabidopsis halleri* expressed different levels and profiles of a specific class of organic metabolites at the accession level as well as influenced the performance of a leaf beetle when growing in metal(loid)-amended and unamended soils.

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## Elucidating the defensive functions of calcium oxalate crystals (raphides) in *Vitis*

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Calcium oxalate crystals are a phylogenetically widespread yet poorly understood plant trait. These crystals are notably diverse in form and location within plants, which suggests variation in their function. While there are several strong hypotheses for the function of these inorganic crystals, such as defense against chewing herbivores and storage of excess calcium, we lack experimental tests of their interactions with arthropods in many plant taxa as well as an understanding of how they interact and correlate with other defensive traits. In this study, we investigate the defense function of calcium oxalate crystals extracted from wild *Vitis riparia* foliage using a no-choice herbivore bioassay with the generalist herbivore *Spodoptera exigua*. We used a factorial experimental design, varying the presence of *Vitis* raphide crystals and *Vitis* secondary metabolites in arthropod artificial diet to test for the existence of a synergism between crystals and plant chemistry. We also manipulated the presence of amorphous calcium oxalate crystals to test for impacts of crystal shape on herbivorous arthropod larvae. Finally, we surveyed a common garden of twenty *Vitis* species and eighteen genotypes of *Vitis riparia* to test for evolutionary correlates of inter and intraspecific variation in raphide and druse crystal expression. We found that *Vitis* raphide crystals did not have an impact on the weight gain, time to pupation, nor the survival of moth larvae, but, contrary to predictions, amorphous crystals did negatively impact the herbivores. We also detected a strong negative effect of grape leaf chemistry on herbivore growth and survival, but no evidence of an interaction between crystals and leaf chemistry. Finally, we saw dramatic variation in the morphology and quantity of crystals in leaves across *Vitis* genotypes and species, from almost no crystal expression to incredibly dense distributions of crystals. Our results suggest that raphide crystals may serve a function other than defense against chewing herbivores in *Vitis riparia*, but that the crystal shape is a determinant of crystal function.

## Arbuscular mycorrhizal fungi promote insect herbivore performance, despite increasing anti-herbivore silicon defences in maize

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Plants have evolved various strategies to cope with adverse climates (abiotic) as well as plant enemies (biotic) and silicon (Si) accumulation can play a vital role for both (1). Most plants are associated with beneficial fungi such as arbuscular mycorrhizal (AM) fungi which can enhance these defences against enemies (2) as well as support Si uptake and accumulation in plants (3). Recent studies highlight a reciprocal relationship between Si and AM fungi, whereby Si supplementation also increases AM colonization (4). This study assessed the tri-partite interaction between plant Si, AM fungi and herbivory by *Spodoptera frugiperda* (Lepidoptera: Noctuidae), a global polyphagous crop pest. Maize (*Zea mays*) was grown with or without Si supplementation, in the absence or presence of AM fungi and half of the plants were exposed to insect herbivores. We evaluated the effectiveness of separate and combined effects of Si and AM fungi treatments at reducing the herbivore performance (relative growth rate (RGR)). Foliar Si concentrations increased with Si supply and were significantly higher in AM colonized plants compared to non-AM plants. While Si reduced insect herbivory, and despite AM fungi-mediated increases in Si concentrations, *S. frugiperda* growth rates were positively affected by AM fungi. The study suggests that silicon defences persisted in AM plants despite AM fungi's positive effects on RGR.

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**Keynote: Crop plants and agriculture: Insect-plant relationships from an applied perspective**

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Understanding plant-insect interactions is fundamental in agriculture for effectively managing pest outbreaks and virus transmission, minimising crop losses in both yield and quality. I will explore the intricate relationship between crop plants and insects, with a specific focus on aphids. This talk will examine the range of species interactions that impact aphid colonization, establishment, and dispersal on crop plants. Harnessing plant microbiomes for insect resistance is a rapidly developing area, yet strong beneficial effects in controlled studies are not always transferred to the field. Knowledge of plant-insect interactions can be exploited to mitigate context-dependency effects reducing natural pest control efforts in field systems. I will explore a range of applied approaches from precision-inoculation of beneficial microbes to landscape management for the recruitment of natural enemies. Moreover, pest insects themselves fight back with strategies to avoid plant defences or resist natural enemies. By integrating these insights, the goal is to identify and exploit community interactions that can collectively enhance crop productivity and resilience, ultimately cultivating a more sustainable agricultural system amidst increasing environmental pressures.

## Digging the diversity of wild potatoes – Metabolomics reveals species- and tissue-specific metabolites promoting resistance against leaf beetles

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Metabolites play a key role in the interaction of plants with insect herbivores and the overall environment and promote plant resistance. In *Solanum tuberosum*, glycoalkaloids are associated with known potato defence mechanisms. Still, the compounds present in current potato varieties are insufficient to prevent severe plant damage and yield loss from pests such as the Colorado potato beetle, *Leptinotarsa decemlineata*, and the potato flea beetle *Epitrix* sp. The lack of effective plant protection products for potato cultivation and the need to reduce their use increase the urgency to identify new plant defence mechanisms and metabolites as metabolic markers for potato resistance.

Wild *Solanum* accessions possess numerous resistances to pathogens and pests while exhibiting enormous metabolic variability, therefore, they represent valuable resources for resistance breeding and the detection of bioactive compounds. However, few studies performed metabolite profiling in *S. tuberosum* or its wild relatives, and knowledge about the metabolic diversity of *Solanum* species and the identity of metabolites active against insect herbivores is scarce.

In the present study, we performed liquid chromatography/tandem mass spectrometry for biochemical screening and feeding assays with the leaf beetles. These experiments revealed resistance in the wild potato species. A new bioactive metabolite was isolated from *S. bulbocastanum*, a wild *Solanum* species that is resistant to numerous potato pests and pathogens. The structure of this Solamin was elucidated by NMR, and its bioactivity against the Colorado potato beetle and *Epitrix* sp. was demonstrated. Further screening of wild and cultivated potato lines by liquid chromatography/tandem mass spectrometry revealed up to 100 structural derivatives of Solamin in other *Solanum* species, even in *S. tuberosum* market potatoes. The accumulation patterns were highly tissue and species-specific, e.g. in *S. bulbocastanum* high concentrations of these compounds were observed in leaves while they were absent in tubers. Considering the observed bioactivity of Solamin, derivatives with a similar structure might have similar resistance-relevant effects. As many of these derivatives occur naturally in *S. tuberosum*, they are promising candidates for further investigation and resistance breeding.

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## **Flower-derived arthropod eDNA reveals community diversity, species abundances and ecological interactions – A case study in bee pollinators**

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Environmental DNA metabarcoding has revolutionized our ability to monitor biological communities. The method has two important advantages over traditional arthropod monitoring approaches. First, eDNA analysis is entirely non-invasive. And second, the approach holds the potential to exhaustively recover true biotic interactions between arthropods and plants. But despite its undeniable promise as a non-invasive tool to study arthropod community and interaction diversity, eDNA metabarcoding from plants has several limitations. One problem of eDNA metabarcoding is its limited capabilities to quantitatively survey organisms. Another problem is the sampling of plant material is usually performed invasively. The plant material, for example flowers, is collected in the field and then washed in the laboratory.

To address these problems, we focus on flower strips in an agricultural landscape in Southwestern Germany, which are visited by an expert for wild bee identification. The expert knowledge provides a very accurate baseline for the true richness and diversity of wild bee species. Sampling was done right after observational data was compiled. We collected two replicate samples for each plant species, one was washed off in the field, while the other was based on harvesting flower heads and washing them in the laboratory.

We show that the arthropod communities recovered by the invasive and non-invasive sampling methods are similar, but especially for monitoring bee species the invasive approach gives a more complete picture as it recovers more species. In addition, we can also make statements about arthropod-plant interactions as we have recovered the arthropod community of different plant species. Here we can show the specificity of different insect species or, conversely, identify species that are not associated with a particular plant species. The overlap of bee species found by the eDNA method and the bee expert is high (43 bee species/58.1%), indicating that eDNA can be used as a monitoring tool for wild bees. We were also able to quantify the bees detected by eDNA. This is a major step forward in the use of eDNA as a monitoring tool. We can also make statements about the flower-bee interaction of the bees using eDNA, as we know which flower species they were found on. It is therefore possible to identify oligolectic and polylectic bee species. Our results highlight eDNA as a tool for monitoring arthropods and studying arthropod-plant interactions.

## Unveiling the role of cover crop mixtures: Implications for maize resistance to pests and pathogens

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Cover crops have been lauded for their positive ecosystem services, but lately researchers have gained interest on how cover cropping can act as a sustainable pest management tool. Recent findings on how individual cover crops impact plant defenses against multiple pests have encouraged our investigation into how cover crop mixtures can aid in mitigating tradeoffs (Davidson-Lowe et al., 2021, Ray et al., 2022). For this study, we evaluated *Zea mays* resistance to fall armyworm (*Spodoptera frugiperda*) and *Fusarium verticillioides* after growing in three different soils: soil legacy of a grass cover crop (triticale: x *Triticosecale*), soil legacy of a brassica cover crop (forage radish: *Raphanus sativus*), and a mixture of soil from those two crop species. We found evidence that the mixture soil might make corn more susceptible to fall armyworm, yet the outcome might be affected by the length at which soil communities are allowed to settle. The investigation suggests that while cover crop mixtures could potentially impact maize resistance to pests, the duration for which soil communities settle might play a crucial role in determining the outcome for the insect. Also unexpectedly, the cover cropping treatments had minimal effects on maize resistance to *F. verticillioides*, but there might be reduced maize root growth in mixture and radish soils, contrary to previous studies. In the future, we hope to characterize defensive chemical responses (phytohormones) in each maize treatment, as well as replicate these experiments in a field study.

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## Can arthropod pests evolutionary adapt to systemic resistance induced by the microbial symbionts of their host plants?

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Environmental concerns about pesticide use in agriculture have stimulated increasing interest in microbially based products for pest control. Among these, entomopathogenic fungi (EPF) have been widely used as foliar sprays, killing arthropod pests through direct interaction with the pest. However, recent studies have shown that these fungi can also provide pest control through indirect, plant-mediated effects, by colonizing the plant as endophytes and inducing systemic resistance against arthropod pests. Whereas EPF are thus a very promising tool for biological pest control, an intriguing - but yet unanswered - question is whether arthropod pests could evolutionarily adapt to overcome such EPF-induced plant resistance.

To address this question, we performed a selection experiment to study adaptation of a notorious pest, the two-spotted spider mite (TSSM) (*Tetranychus urticae*) to resistance induced by the entomopathogenic fungi *Metarhizium brunneum* and *M. robertsii* in tomato. In previous studies we have shown that strains of both fungi can significantly reduce the population growth of spider mites on tomato plants. We initiated a selection experiment by establishing eight independent spider mite lineages from a single base-population. Spider mites from each lineage were then selected for multiple generations on either control plants, plants inoculated with *M. brunneum*, or plants inoculated with *M. robertsii*. After four and twenty generations, bioassays were performed to study the performance (oviposition and population growth) of the evolved spider mite lineages on control and fungus-inoculated plants.

We present the results of this experiment to address the following questions. (1) Do spider mites selected on fungus-inoculated plants evolve adaptations to fungus-induced resistance, i.e. do they perform better on fungus-inoculated plants than mites from control lineages? (2) Do spider mites selected on *M. brunneum*-inoculated plants evolve cross-adaptation to resistance induced by *M. robertsii* and vice versa? (3) Does adaptation to fungus-induced resistance incur fitness costs expressed as a lower performance on control plants compared to mites from control lineages? Overall, our results provide proof-of-concept that arthropod pests can adapt to resistance induced by microbial symbionts of their host plants. We discuss the results in the light of management practices for EPF-based biocontrol to prevent loss of their efficacy due to pest adaptation.

## Dual function of omnivorous predators in biocontrol: As bodyguards and plant defence vaccinators

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Omnivorous predators have been increasingly used in biocontrol in greenhouse crops. As omnivores, they can predate on herbivores and feed on plants. Their role as predators has been studied extensively, whereas the role of plant feeding has been ignored until recently. It has been shown that plant feeding by different omnivores can induce plant defences that decrease the performance of several herbivores and production of plant volatiles that attract natural enemies sharing the same prey. Thus, omnivores can serve dual function in biological pest control: as predators and plant defence vaccinators. However, most study have been performed in greenhouse crops, and little is known whether and how omnivores alter herbivore performances through inducing plant defences in other systems, such as open field crops. Here, we studied how the plant responses induced by the omnivorous predator *Orius laevigatus* alter the performance of five herbivores (3 chewing and 2 sucking insects) in *Brassica nigra*. In addition, we investigated whether the omnivore-induced responses influence the oviposition preference of females of three chewing herbivores. We found that the performance of three herbivores was decreased on plants previously exposed to the omnivore for 4 days than on clean plants. The fresh weights of *Pieris brassicae* and *Plutella xylostella* caterpillars and the numbers of *Brevicoryne brassicae* aphids fed on omnivore-exposed plants were significantly lower than the ones on clean plants. Whereas the fresh weight of *Mamestra brassicae* and the numbers of *Myzus persicae* aphids were similar on both plants. Furthermore, females of *P. brassicae* preferred clean plants to omnivore-exposed plants. After 2 hours exposure to both plants, these females oviposited significantly lower numbers of eggs on omnivore-exposed plants than on clean plants. Females of *P. xylostella* and *M. brassicae* laid fewer eggs on clean plants than exposed plants, though the differences were not statistically different. Our findings indicate that omnivorous predators induce plant defences that influence performance and preference of various herbivores in the open field crop *B. nigra* system, potentially decreasing pest pressure under field conditions.

**Keynote: Microbes modulate plant performance and responses to global change**

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Microbes essentially contribute to achieving the UN's Sustainable Development Goals for example by helping plants adapting to global change components. Our data show that stability of ecosystem functions depends on the variability of plant species-specific responses to global change components, and that microbes have the potential to modulate these responses. These beneficial effects are achieved by rapid alterations of the plant's microbiome that mediate the plants' phenotype and performance as well as interactions with pollinators and herbivores. At the same time local and regional microbial diversity, the prerequisite for dynamic alterations of microbiomes, is declining due to direct global change effects, the homogenization of microbial communities across larger scales, as well as cascading effects of macrobiological diversity decline. A reduced microbial diversity and mobility between ecosystem constituents may thus threaten the stabilizing role of microbiomes calling for microbiome stewardship and the conservation of microbial biodiversity.

# Poster Presentations

P1-01

## **Coevolution in a changing world: The impact of temperature on reciprocal selection in an experimental plant-pollinator-herbivore system**

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The geographic mosaic of coevolution predicts reciprocal selection, the first step in coevolution, to vary with changing biotic and abiotic environmental conditions. Studying how temperature affects reciprocal selection is essential to connect effects of global warming on the macroevolutionary patterns of coevolution to the ecological processes underlying them. In this study, we first established that the interaction between fast cycling *Brassica rapa* plants and its butterfly “pollinating herbivore” *Pieris rapae* had fitness consequences for both interactors, a requisite for reciprocal selection. Next, we grew *B. rapa* plants in two temperature environments (ambient and hot) and exposed them to *P. rapae* butterflies for flower visitation and oviposition. We measured the phenotypes of plants and butterflies, their interactions and fitness, which we used to calculate reciprocal selection. Elevated temperature induced extensive plasticity in flowering plant traits, changed butterfly behaviour, and altered caterpillar growth and development. Thereby, elevated temperature changed the plant fitness outcome of butterfly visitation and herbivory to become more antagonistic, as well as the fitness of the butterflies. We found a network of traits involved in reciprocal selection, and this network was different between the ambient and hot environment. We provide experimental evidence that variation in an abiotic factor, elevated temperature, changes reciprocal selection between a plant and its pollinating herbivore. Uncovering the underlying mechanisms by which global warming changes reciprocal selection between organisms will help better predict its consequences for coevolution.

P1-02

## Thermal performance of fitness-related traits in competing parasitoids

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The performance of ectotherms strongly correlates with environmental temperature. The way temperature affects their performance has a strong impact on their realised fitness and their biotic interactions. In a thermally heterogeneous environment, interacting species with dissimilar thermal performances may cohabit by using different thermal micro-sites while species with close thermal performances may face strong competition for similar thermal micro-sites. Indeed, closely interacting species sharing the same resource and habitat are likely to share close thermal performance, and the preference for similar thermal micro-sites would increase competition level. Our work focus on the impact of temperature on the competition between two parasitoid species, *Dinarmus basalis* and *Eupelmus vuilleti*, in which females compete to access their common hosts, larvae of *Callosobruchus maculatus*, a crop pest of *Vigna unguiculata*. These species co-exist in western Africa, in a tropical savanna climate characterized by high temperatures (40-45°C). We measured the temperature dependence of several traits related to their fitness (oviposition rate, juvenile survivorship, developmental time, fecundity and size at emergence) and used the established thermal performance curves (TPC) as tools to predict their realised performance and anticipate the risk of increased competition that may result. We predicted that both parasitoid species have a similar thermal optimum, over 30°C with a relatively narrow thermal breadth. These TPCs would be coherent with the hot and stable environment they encounter in Africa. Our first results are in accordance with our predictions, as the two species present a thermal optimum around 30-35°C and a thermal breadth of 15°C from 25°C to 40°C. The similarity in their thermal performance implies that these two parasitoids should be competing strongly for their resource (host) as they target the same temperature micro-sites. The narrow thermal breath may imply a striking decrease in performance in the weaker competitor as it could be pushed towards sublethal temperatures.



P1-03

## **Egg priming of plant anti-herbivore defenses in a warming world: A case study in a Lepidoptera-Brassicaceae system**

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Mitja Remus-Emsermann, Vivien Lortzing

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Defenses of many Brassicaceae plants are primed by the oviposition of the Large White butterfly, *Pieris brassicae*, an economically relevant pest of cabbage crops. Priming of plant defenses leads to impaired development and higher mortality of larvae, resulting in less plant damage. Previous studies under standardized laboratory conditions at 20 °C showed that *Arabidopsis thaliana* needs to be exposed to *P. brassicae* eggs for at least five days to prime its defenses against conspecific larvae. However, the hatching time of *P. brassicae* eggs is temperature-dependent, and warmer temperatures reduce the duration of plant exposure to eggs due to earlier hatching. Considering that temperatures are expected to increase by approximately 3.0 °C by the end of this century in continental Europe, the question arises how temperature increase affects the interaction between *P. brassicae* and its host plants. We aimed to answer whether this will have an effect on the egg-mediated primability of anti-herbivore defenses. We hypothesize that plants are able to “predict” the time of the temperature-dependent *P. brassicae* larval hatching and adjust their defense responses accordingly. To test this hypothesis, we treated *A. thaliana* with eggs at different temperatures. We assessed larval and plant performance as well as plant gene expression of egg-primable genes and phytohormone levels. Experiments were conducted at 16, 20 and 23 °C, the average temperatures of late spring, early and late summer in Berlin, Germany, when *P. brassicae* occurs in nature. We found that plants kept at 16 °C exhibited egg-priming responses after ten but not five days, while at 23 °C, no priming effect was detectable. Our results highlight the importance of understanding the temperature dependence of plant – insect pest interactions for our future crop management in the context of global warming.

P1-04

## Responses of a fundamental functional trait “body size” of ground-dwelling beetles to large-scale environmental changes.

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Large-scale environmental changes caused by global climate changes, such as global warming or landslides, have profound impacts on biodiversity around the world. Recently, a biological response of body size changes to large-scale environmental changes is getting attention. Particularly, the body size reduction to global warming is being reported from numerous species, suggesting the phenomenon may be a third universal response to climate warming. Because body size is a fundamental functional trait involved in adaptation and population dynamics through ecological processes such as reproduction and resource acquisition, revealing body size responses to large-scale environmental changes is critical for an understanding the organism’s adaptation under climate changes.

In this study, we focus on the body size responses to two large-scale environmental changes, landslides and global warming. Precipitation-induced landslide is one of the large-scale environmental changes, which are predicted to increase under changing climate. However, an understanding of how insect community properties including species body size shift following landslides remains limited because replicated research involving landslides, which are large-scale disturbances with stochastic natural causes, is difficult. To tackle this issue, we established 12 artificial landslide sites, each 35 m × 35 m, and 6 undisturbed sites and collected ground-dwelling beetles. To investigate the relationship between body size and global warming, we measured the body size of *Synuchus* sp. which is one of the species collected from landslide sites, using specimens collected along latitudinal cline in Japan.

We found that the structures of landslide and undisturbed communities were totally different. There were no significant differences in species diversity between undisturbed and landslide communities, however, among-site variability in species composition was much greater at landslide sites. The body size of beetles collected in landslide sites was significantly smaller than in undisturbed sites, suggesting colonization by beetle species with a high dispersal capacity same as previous reports. The body size of *Synuchus* sp. was smaller in lower latitude, suggesting that body size of this species may decrease in warmer region following the Bergmann’s rule. We would like to discuss about community assembly process of landslide communities and the body size reduction of *Synuchus* sp. to global warming.

P1-05

## Climate change impacts on mealybugs vectoring grapevine leafroll disease

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Climate change impacts crop plants, plant pathogens, and their insect vectors, adding abiotic stress to the triangle of plant-virus-vector interactions. Grapevine is among the most widely grown fruit crops worldwide and grapevine leafroll disease (GLRD) is probably the most widespread viral infection in vineyards. It is transmitted through infected propagation material and insect vectors, such as the vine mealybug *Planococcus ficus* (Signoret). Knowledge of climate change impacts on mealybugs in general or as virus vectors, in particular, is scarce. Similarly, we lack information on grapevine plant defence against pathogens under climate change conditions. Plant-virus-vector interactions are usually very species-specific hence conclusions cannot be derived from other pathosystems. It is therefore unclear how climate change influences the plant-virus-vector interactions of GLRD. Here, we aim to fill this gap by evaluating i) life-history parameters of the insect vector, *Planococcus ficus*, under elevated CO<sub>2</sub> (eCO<sub>2</sub>) in greenhouse and field experiments (Schulze-Sylvester & Reineke, 2019) and ii) the impacts of elevated temperature (eT) and elevated CO<sub>2</sub> (eCO<sub>2</sub>) on *P. ficus* and its ability to vector GLRD. At the same time, we also focused on amino acids and defence-related plant parameters (gene expression and phenols). The greenhouse experiments with an eCO<sub>2</sub> showed a significant increase in survival rates, a strong trend towards declining body size, and an increasing fecundity of female mealybugs, while fertility and development time did not change. We also found differences in disease transmission rates and certain phenol levels. The obtained data will serve to close basic knowledge gaps on individual species, their interactions, and the disease they cause in current and future climate scenarios. Enhancing the knowledge of the impacts of climate change on economically important plant diseases and their insect vectors is crucial to strengthen the adaptive capacity of crop production and ensure sustainable viticulture.

Schulze-Sylvester, M., & Reineke, A. (2019). Elevated CO<sub>2</sub> Levels Impact Fitness Traits of Vine Mealybug *Planococcus ficus* Signoret, but Not Its Parasitoid *Leptomastix dactylopii* Howard. *Agronomy*, 9(6), 326. <https://doi.org/10.3390/agronomy9060326>

P1-06

## **Legacy effects of drought-driven premature defoliation modulate phytochemical profiles of European beech with subtle consequences for leaf herbivory**

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Extreme droughts can have long-lasting effects on forest community dynamics and species interactions. Yet, our understanding of how drought legacy modulates ecological relationships is just unfolding. We tested the hypothesis that leaf chemistry and herbivory show long-term responses to premature defoliation caused by an extreme drought event in European beech (*Fagus sylvatica* L.).

For two consecutive years after the extreme European summer drought in 2018, we collected leaves from the upper and lower canopy of adjacently growing drought-stressed and unstressed trees. Leaf chemistry was analyzed and leaf damage by different herbivore feeding guilds was quantified.

We found that (1) drought had lasting impacts on leaf nutrients and on specialized metabolomic profiles. However, drought did not affect the primary metabolome. (2) Drought-related phytochemical changes affected damage of leaf chewing herbivores whereas damage caused by other herbivore feeding guilds was largely unaffected (3) Drought legacy effects on phytochemistry and herbivory were often weaker than between-year or between-canopy strata variability.

Our findings suggest that a single extreme drought-event bears the potential to long-lastingly affect tree-herbivore interactions. Drought legacy effects likely become more important in modulating tree-herbivore interactions since drought frequency and severity are projected to globally increase in the coming decades.

P1-07

## Life stage- and sex-specific responses to nutritional stress in a holometabolous insect

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Over the course of their lives, organisms experience different stresses that shape their phenotype. Individuals may deal with stress differently, also depending on the life stage at which they experience it and on their sex. One of these stresses is nutritional stress, such as limitation in food. However, little is known about how responses differ between individuals experiencing nutritional stress early versus later in life or repeatedly, particularly in species with distinct ontogenetic niches. Moreover, females and males may respond differently due to distinct needs. The turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae), is a holometabolous herbivore, which demands different food plant families across its lifespan. Larvae feed on leaves and flowers of various Brassicaceae, while the adult sawflies take up nectar from, for example, Apiaceae. Here, we examined effects of starvation, experienced at different sensitive phases, on life-history traits, behaviour and metabolic traits. Therefore, we set up four distinct starvation regimes. Individuals experienced either no starvation, periodical larval starvation, adult starvation or starvation periods in both larval and adult life. Larvae exposed to starvation had a prolonged developmental time but reached a similar adult body mass as non-starved individuals, suggesting the ability to compensate until adulthood. Adult starvation led to a significantly reduced body mass in females only. The adult behaviour, measured as activity, was not impacted by larval starvation, but, by trend, adult starvation led to a higher activity in both sexes. Moreover, individuals starved as larvae could reach similar carbohydrate and lipid contents than non-starved individuals, potentially by building up energy reserves, while adult starvation or starvation in both stages led to reduced energy reserves, here mostly in males. In summary, individuals show differences in niche conformance, with life stage- and sex- specific responses to nutritional stress.

P1-08

## Salinity stress and aphid infestation on sugar beet plant chemistry: Impacts on aphid-parasitoid interactions

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Climate change can lead to increased soil salinity due to salt water intrusion and excessive irrigation. High salinity levels alter the chemical composition of plants, yet little is known about how this affects the interactions between plants, herbivorous insects, and their natural enemies. Our study comprehensively investigated how herbivory by aphids (*Aphis fabae*) and two magnitudes of salinity alter the morphology, physiology, and phytochemistry (central metabolites, phytohormones) of sugar beet (*Beta vulgaris*). Additionally, effects of salinity and aphid herbivory on the emission of volatile organic compounds (VOCs), and preferences and performances of parasitoids (*Aphidius colemani*) were investigated. Elevated salinity levels were found to adversely affect sugar beet growth in terms of biomass, leaf size and photosynthetic capacity. Aphid infestation did not further influence these parameters, except for photosynthetic capacity, as high salinity and infestation led to lower chlorophyll fluorescence. Both salinity and aphid herbivory significantly changed the concentrations of several plant hormones, such as abscisic acid, 12-oxophytodienoic acid, jasmonates, and salicylic acid. Central metabolome analysis showed that increasing salinity stress increased concentrations of amino acids, organic acids, fatty acids, and sugar metabolites. Salinity stress further resulted in smaller sized aphids and lower reproduction rates. On the third trophic level, enhanced salinity indirectly decreased parasitoid fitness as evidenced by lower emergence rate, altered sex ratio, and reduced body size. Salinity stress further resulted in a decreased overall quantity and diversity of volatile organic compounds (VOCs), but without compromising parasitoid attraction to aphid-infested plants. Our findings demonstrate that salinity, aphid herbivory, and the combined stresses alter the physiology of sugar beet plants in non-additive ways, defying prediction. These alterations cascade through trophic links, leading to detrimental effects on both *A. fabae* and its parasitoid *A. colemani*. Under the tested conditions, the impacts of abiotic and biotic stresses on the plant appear asymmetric, with salinity stress exerting stronger negative effects.

P3-01

## Identification and characterization of O-methyltransferase genes involved in the biosynthesis pathway of benzoxazinoids in wheat

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In nature, plants encounter various biotic and environmental stresses. As a result, plants produce arrays of specialized metabolites to reduce the damage and adjust to the stresses. Benzoxazinoid (BXD) is a class of specialized metabolites that has broad functions in plant defense mechanisms. Although the BXD pathway has been studied for decades mostly in maize, the genes involved in the biosynthesis pathway and their roles are partially elucidated in wheat. The aim of the study was to identify and characterize O-methyltransferase (OMT) genes involved in BXD metabolism. A list of novel OMT candidate genes that might potentially be involved in the biosynthesis pathway of BXDs was obtained using different approaches, including network analysis, QTL-mapping, RNA sequencing, and comparison to ortholog genes from maize and rye. We showed the function of the candidate genes for wheat BX7 enzyme in planta using a virus-induced gene silencing (VIGS) system. Furthermore, the enzymatic activity of the candidate genes was studied by co-expression in *Nicotiana benthamiana*. Additionally, the role of the candidate gene in wheat defense against insects was tested using VIGS-silenced plants. Overall, the research uncovers one of the OMT enzymes involved in the biosynthesis of BXD in wheat. Moreover, the study provides broad impacts on the roles of BXDs in wheat plants' defense/resistance mechanisms against insects that may contribute to wider plant breeding, promoting natural pesticides, and improving the sustainability of agricultural plants.

P3-02

## Wheat's altered diurnal rhythm in response to *Rhopalosiphum padi* aphid infestation

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Wheat is an important crop, used for both human consumption and livestock feed. Herbivore pests can cause massive damage and thereby reduce grain yield. Nevertheless, not much is known about the effects of pest infestation on the plant's diurnal rhythm.

Here, we present a time series analysis to expose the effect of aphid infestation on wheat's natural diurnal cycle.

A total of 15,366 rhythmic genes were identified in wheat under normal conditions. However, under infestation, 5,682 genes lose their rhythmic pattern. Moreover, under infestation, 5,203 genes gained rhythmic activity. These genes that gained rhythmic activity were enriched for stress-related GO terms and were highly enriched for motifs of the WRKY transcription factor family.

Core circadian genes maintained their pattern under infestation, however, differently expressed genes were enriched for their motifs. This suggests that circadian genes play a defensive role under aphid infestation, a role that has been previously reported.

Using untargeted metabolomics, we identified rhythmic metabolomic changes under infestation. Several monoterpenoids gained rhythmic activity under infestation, while saccharides retained their rhythmic activity when exposed to aphids.

Transcriptomics and metabolomics integration revealed that the rhythmic loss of genes was related to the response to oxidative stress. Meanwhile, genes that gained rhythmicity were enriched for those related to the pentose phosphate pathway.

These findings further enhance our understanding of plant-insect interactions and could help guide future studies focused on time optimized plant protection.



P3-03

**The bittersweet story: A complex role of jasmonic acid in *Solanum dulcamara*'s wound nectar production**Lu Li, Tobias Lortzing, Anke Steppuhn*University of Hohenheim, Stuttgart, Germany*

Nectar is a sugary plant secretion that attracts beneficial animals and is usually released from specific structures, the nectaries. While flowers produce nectar (FN) for pollination, extrafloral nectar (EFN) can be secreted from almost all plant parts for plant defence by attracting natural enemies of herbivores, primarily ants. In several plant species, EFN was shown to be induced by the phytohormone jasmonic acid (JA) which is well-known to induce various plant defence responses to herbivory. The bittersweet nightshade *Solanum dulcamara*, despite bearing no nectaries, produces a special form of EFN directly from wounds caused by herbivores. This wound nectar (WN), just like EFN, can function as indirect defence by attracting ants and is JA-inducible. We aim to investigate the mechanisms underlying this WN secretion in *S. dulcamara* and its regulation. We studied different conditions that increase WN secretion such as repeated wounding and drought to find potential candidate genes transcriptionally correlated with variable WN secretion levels. We created CRISPR-Cas9-mediated knock-out mutants of candidate genes such as sweet sugar transporter and a JA-biosynthesis gene *Lox3*. Both are putative homologues of characterised genes in related plant species, *sweet9* mediates FN secretion in several plant species including tobacco and the tomato *LoxD* is responsible for the rapid JA burst in response to wounding. We compared WN production between these mutants and wild-type under different conditions, such as mechanical wounding, drought, and exogenous methyl jasmonate. We further examined, whether the *Lox3* gene also confers induced defence to herbivores and determined its consequences for insect performance and leaf chemistry. WN secretion was not reduced in the *SWEET*-mutant suggesting that this transporter is not involved. The *Lox3*-mutant were as expected deficient in the wound-induced production of JA and herbivory-induced defence compounds. Performance of a generalist lepidopteran herbivore was increased on this mutant, confirming the role of *Lox3* in anti-herbivore defence. Unexpectedly, the *Lox3*-mutants secreted significantly higher WN comparing to wild-type. However, the combination of drought stress and wounding treatment can further increase WN in *S. dulcamara* independent of *Lox3*, suggesting complicated crosstalk among plant phytohormones. These findings reveal that JA is involved in WN secretion in a more complex manner than as a simple positive regulator.

P3-04

### Response of the chemical diversity of *Brassica rapa* to reduced pollinator availability

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There is evidence of a worldwide reduction in the richness and availability of pollinators. Plants must develop chemical adaptations to thrive in these conditions. Yet, a surprising gap emerges when we question the adaptive response of chemical diversity to the decline in pollinators. We explored evolutionary changes in chemical traits after selecting *Brassica rapa* for six generations under various levels of pollinator access. Primary and specialised metabolism was scrutinised in leaf and flower samples to explore the response of *B. rapa* from chemical diversity indices to the metabolite. Significant variation in richness and functional Hill diversity was observed in flowers. Next, generalised linear models were used to unveil a set of leaf and flower metabolites predicting pollinator access with more than 80% accuracy. This chemical toolbox included primary and specialised metabolites, some of which also referred to the redox state. Overall, these results represent a unique advance in our understanding of how plants evolve in response to reduced pollinator availability. In addition, this study supports the value of predictive metabolomics for studying and understanding ecological processes, which would improve prediction of the response of ecosystems to global change.

P3-05

**Distribution of a non-proteinogenic amino acid in Brassicaceae tissues: Implications for defense against phytophagous insects**

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Insects pose a significant threat to plants, which defend themselves through several morphological and biochemical traits. Among these defense mechanisms, the role of non-proteinogenic amino acids, such as S-methyl-L-cysteine sulfoxide (SMCSO), remains largely unexplored. A previous study has shown that the distribution of SMCSO in plant reproductive tissues is consistent with the predictions of the Optimal Defense Theory (ODT) and may serve as a defense against phytophagous insects. To further investigate the evolution of SMCSO as a defense, we characterized its spatial distribution in the plant tissues of twelve Brassicaceae species and assessed its effects on the feeding behavior of both generalist and specialist phytophagous insects. Twelve cultivated and wild species along the Brassicaceae phylogeny were grown and their roots (primary and secondary), leaves (young and old), and inflorescences were harvested for SMCSO quantification. Using both specialist (*Delia radicum*, *Psylliodes chrysocephala*, *Ceutorhynchus assimilis*) and generalist (*Pachnoda marginata*, *Spodoptera littoralis*, *Brassicogethes aeneus*) herbivores feeding from the different plant parts, we conducted feeding tests on artificial diets supplemented with physiological concentrations of SMCSO. Our experimental design aimed to test the hypothesis that generalist herbivores would be deterred by SMCSO, while specialists would show variable responses that may include stimulation or no effect. We found variation in SMCSO levels across species and tissues. Among species producing SMCSO, its distribution within the plant was consistent with ODT predictions, with higher concentrations in reproductive tissues compared to vegetative tissues, and higher levels in young leaves and primary roots compared to old leaves and secondary roots, respectively. We found that physiological concentrations of SMCSO had a deterrent effect on the feeding of generalist herbivores, with the degree of deterrence depending on concentration. The effects on specialist herbivores varied, with a stimulant effect on *D. radicum*, no effect on *C. assimilis* and a deterrent effect on *P. chrysocephala*. These results support the defensive role of SMCSO against phytophagous insects and highlight the potential of non-proteinogenic amino acids as key players in plant-insect interactions. Future research should focus on the inducibility, biosynthesis, and transport mechanisms of SMCSO to further elucidate its role in plant defense.

P3-06

**Differential gene expression toward species of *Aristolochia* impairing the performance of the specialist troidini butterfly *Battus polydamas* (Troidini: Papilionoidea)**Karina Brandao<sup>1</sup>, Julia Teresa<sup>2</sup>, Clecio Klitzke, Marcelo Brandao<sup>2</sup>, Jose Trigo<sup>2</sup><sup>1</sup>Leibniz Institute for the Analysis of Biodiversity Change, Hamburg, Germany<sup>2</sup>State University of Campinas, Campinas, Brazil

The neotropical swallowtail butterfly *Battus polydamas* is a specialist on *Aristolochia* (Aristolochiaceae). These plants are rich in natural products such as terpenoids, lignans,  $\beta$ -phenylethylamines ( $\beta$ PEA), aporphine and isoquinoline alkaloids, and aristolochic acids (AAs). Larvae of *B. polydamas* sequester some of these compounds, such as AAs, and transfer them to adults through the pupae. AAs are considered defensive compounds against natural enemies, however, the amount of AA in the larvae's diet has an effect on their performance, which may mean a cost to eating on AA-containing leaves. In the present study we evaluated the performance of *B. polydamas* larvae fed from 1st instar through pupation on two host plants with different chemistry composition, *A. galeata* (which has several diterpenes) and *A. gigantea* (which has acyclic monoterpenoids and sesquiterpenoids, but no diterpenoids or AAs). Differential gene expression as response to different larval host plants was evaluated in three biological replications of midguts and fat body tissues of six 5th instar larvae. We found significant differences in the survival of larvae feeding on the two host plants, the survival in *A. gigantea* being significantly higher than survival in *A. galeata* (GLM binomial, likelihood ratio test,  $df = 1$ ,  $\chi^2 = 76.082$ ,  $P < 0.001$ ). In *A. gigantea*, 55% of the larvae persisted until pupation, while none of the larva feeding on *A. galeata* survived. Time of larval development on *A. gigantea* was  $16.2 \pm 0.4$  days. 951 GOs identified by their molecular function were upregulated in the midgut of larvae fed on *A. gigantea*, while 2203 were downregulated. Upregulated GOs include genes encoding for ribosomal proteins, superoxide dismutase, P450s, UGTs, glutathione S-transferase and many proteases. Downregulated GOs comprise genes encoding for ribosomal proteins, protein farnesyltransferase (involved in diterpenes metabolization, and hypothesized to function in a pathway to synthesize predator-repelling terpenes secreted by the osmeterium of Papilionidae butterflies), Phosphomevalonate kinase, Dolichyl-phosphate-mannose-protein mannosyltransferase 4 and O-glucosyltransferase (possibly involved in AAs metabolization). As expected, larvae of *B. polydamas* were strongly influenced by host plants exhibiting different concentrations of AAs, with higher concentrations leading to worse larval performance on key fitness components, such as life cycle performance attributes and larval survival.

P3-07

## Host plant adaptation in Plutellid moths - Do glucosinolate sulfatase specificities match host plant glucosinolate spectra?

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The glucosinolate-myrosinase-system is a well-studied plant defense system against pathogens and herbivores. It occurs in plants of the Brassicales and is characterized by an immense structural diversity. One of the most damaging pests of Brassicaceae crops, the diamond back moth (*Plutella xylostella* (Lepidoptera: Plutellidae)), overcomes the glucosinolate-myrosinase defense system by larval gut-expressed glucosinolate sulfatases (GSS). Other species of the Plutellidae also use glucosinolate-containing plants as hosts, but with a remarkable variation in host plant range. Based on the hypothesis that Plutellidae species generally employ sulfatases to overcome the glucosinolate-myrosinase system, we wanted to test if sulfatase substrate specificity together with host plant glucosinolate profile determine host plant specificity and host range of these insect herbivores.

To assess host plant glucosinolate diversity, we collected leaf samples of different Brassicales in Germany and France and characterized the glucosinolate spectra by HPLC-DAD and LC-MS analyses. In parallel, we generated sequence information of selected Plutellid species to identify putative GSS genes and to reconstruct ancestral sulfatase sequences. We expressed about 40 candidate GSS enzymes heterologously in Sf9 cells and tested their activity towards a mix of 21 glucosinolates or a chromogenic arylsulfatase substrate.

Each of the seven analyzed Plutellidae species (representing four different genera) expresses multiple GSS. Their classification as GSS1 and GSS3 based on phylogenetic analysis corresponds to their substrate specificities. Within the GSS types, the substrate specificities of enzymes from different Plutellidae species vary less than expected. Combining the activity of all GSS of one species, the preferred substrates do not reflect the glucosinolates of all its host plants. This indicates that GSS are only partly adapted to the specific glucosinolates occurring in the corresponding host plants. Furthermore, the GSS substrate range of polyphagous species does not significantly differ from that of oligo- or even monophagous species. GSS seem to have a monophyletic origin within the Plutellidae. Characterization of enzymes encoded by reconstructed ancestral GSS genes demonstrates functional changes over their evolutionary history in agreement with a specialization towards glucosinolates as substrates.

P3-08

**Gregarines do not play a role in metabolism of glucosinolates by a leaf beetle**Alessa Barber, Jeanne Friedrichs, Caroline Müller*Bielefeld University, Bielefeld, Germany*

The leaf beetle *Phaedon cochleariae* is a specialist on Brassicaceae plants, which utilize the myrosinase-glucosinolate-system to defend themselves from herbivores. Myrosinases hydrolyze glucosinolates, forming toxic products such as isothiocyanates and nitriles. Larvae and adults of *P. cochleariae* are known to detoxify intermediate breakdown products of glucosinolates by conjugation with amino acids. This detoxification step also occurs when insects feed on plants lacking internal myrosinases. In their guts, *P. cochleariae* host the gregarine species *Gregarina cochlearium*. Gregarines are unicellular organisms that can be viewed on a parasitism-mutualism-spectrum. We investigated whether these gregarines contribute to the metabolism of glucosinolates in *P. cochleariae* by offering leaves of watercress (*Nasturtium officinale*, Brassicaceae, including mainly the benzenic 2-phenylethyl glucosinolate and myrosinases) or pea (*Pisum sativum*, Fabaceae, lacking both) to larvae either infected or not infected with gregarines. Leaf discs were treated either with the aliphatic 4-pentenyl glucosinolate or the indole 1-methoxy-3-indolylmethyl glucosinolate. After consumption, larvae and feces were analyzed via UHPLC-QTOF-MS/MS and samples scanned for possible metabolites. Breakdown metabolites of both 2-phenylethyl and 4-pentenyl glucosinolate were detoxified by conjugation with aspartic acid, while breakdown metabolites of 1-methoxy-3-indolylmethyl glucosinolate were conjugated with glutamic acid. These conjugates were found independent of gregarine infection and presence of host plant myrosinases. Thus, another gut symbiont belonging to bacteria or fungi may aid in the metabolism of glucosinolates in this leaf beetle species.

P3-09

## **New methods against old enemies - Rearing and testing of cabbage stem flea beetles under controlled conditions**

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Rapeseed is exposed to a variety of harmful pathogens and pests in the field. The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala*, is a very important economic pest of winter rapeseed and other *Brassica* species in Europe. Adult beetles damage the plant by feeding on cotyledons, leaves and stems and can lead to complete crop failure under high infestation pressure. Larvae penetrate the plant and drill through the leaf petioles and stems. Both adult and larval stages are harmful and result in reduced plant vigor, reduced overwinter survival and ultimately reduced yield.

To better understand of this insect-host-plant interaction, it is important to conduct detailed research. The fact that field trials are seasonal, depend on varying levels of natural infestation, and can often only be conducted with a small number of replicates, is especially challenging for plants with such a long growing season such as rapeseed. Laboratory tests therefore have the great advantage that they can be carried out in larger numbers and regardless of the season. This is of value for detecting small differences between related varieties and genotypes of rapeseed plants. There is therefore a great need to develop reliable and fast laboratory test-systems that allow smallest changes in the insect-plant interaction, such as changes in morphology or content of secondary metabolites, to be detected.

For this purpose, various experimental laboratory methods have been set up and experimental designs evaluated. Using video recordings, high-throughput screening and molecular biology approaches, smallest changes in the interaction between rapeseed host plants and the flea-beetle insect herbivore will be investigated.

P3-10

## Oviposition priming of the wild tobacco's tolerance response to larval feeding

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Insect egg deposition can serve as stimuli which indicate future herbivory for the plant, as it often precedes the attack of herbivorous larvae. Various plant species improve their induced defence against herbivorous larvae when they previously perceived insect egg deposition as a priming signal. Defence priming is assumed to be an adaptive strategy by which the plant optimizes cost-benefit trade-offs of induced defence. We investigated the metabolic state and fitness consequences of oviposition priming and herbivory on the wild tobacco, *Nicotiana attenuata*. This plant induces more defensive phenylpropanoid-polyamine conjugates in response to feeding by lepidopteran larvae when it had been previously oviposited which results in a higher resistance towards a generalist but not the specialist lepidopteran herbivore *Manduca sexta* (1,2). As the attack of *M. sexta* is known to induce tolerance responses, such as transient carbon allocation to the roots that can result in a prolonged reproductive phase (3), we asked whether oviposition priming may also affect such tolerance responses. Therefore, we exposed plants in a full-factorial setup to oviposition and larval feeding by *M. sexta*, followed by a total aboveground shoot removal. We determined growth parameters and the production of reproductive units such as flowers, capsules and seeds as fitness estimates of the re-grown plants. Additionally, we measured the contents of soluble sugars and aromatic amino acids as well as transcript levels of assimilate transport-related genes in the roots and shoots to monitor the carbon allocation. These experiments revealed that previous oviposition in combination with larval feeding can increase the fitness of regrown plants. Thus, oviposition-mediated defence priming may increase tolerance responses to larval feeding.

(1) Bandoly et al., 2015, Plant J., 83(4)

(2) Bandoly et al., 2016, Plant Cell Environ., 39(4)

(3) Schwachtje et al., 2006, PNAS, 103(34)



P3-11

### Differential induction of *Medicago truncatula* defense metabolites in response to rhizobial symbiosis and pea aphid infestation

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Legumes establish symbiotic relationships with nitrogen-fixing bacteria, enabling them to grow in nitrate-poor soils. Legumes are also attacked by plant suckling insects such as the aphids. In addition to N furniture, plant-microbe symbioses can induce plant systemic defence reactions against bioagressors. The aim of this work was to investigate whether nitrogen-fixing symbiosis (NFS) can prime plant defence against the pea aphid *Acyrtosiphon pisum* in the leguminous *Medicago truncatula*. We analysed the expression of defence genes and metabolite modification in both NFS and nitrate-fed (non-inoculated, NI) plant leaves with/without aphid infestation. Accumulation of primary and secondary metabolites, measured by GC-MS and LC-MS, were modulated by both NFS and aphid infestation. We found that 62 defense related metabolites such as salicylate, pipecolate, gentisic acid and several soluble sugars were differentially regulated by aphid infestation in both NFS and NI conditions. We also observed that 19 metabolites including triterpenoid saponins were specifically accumulated in NFS conditions upon aphid infestation.

Gene expression analysis showed that aphid-infested plants exhibited significantly higher expression of Pathogenesis Related Protein 1, a marker for the salicylic acid pathway, under both NFS and NI conditions. Proteinase Inhibitor, a marker of the jasmonic acid pathway, was also induced by aphid infestation, but with significantly higher expression in NFS conditions compared to NI conditions. We also observed a significant induction of several genes involved in defence metabolite pathways in infested plants showing a genetic regulation of the defence mechanisms. Our data suggest that NFS may be developed in the future as a tool for legume pest biocontrol.

P4-01

### **Benzoxazinoids: regulation of a single class of specialized metabolites involved in adjusting to multiple stressors**

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Plants in nature are continuously challenged by diverse insect herbivores. To reduce and arrest the damage insects cause, plants synthesize defensive molecules that dynamically accumulate depending on temporal and spatial factors and stressors. Benzoxazinoids (BXDs) are specialized metabolites abundant in staple crops such as maize and wheat. Although their biosynthesis has been studied for decades, the regulation of this pathway remains mostly unknown. We generated transcriptomic data of wheat leaves infested with aphids and caterpillars and identified the MYB31 transcription factor co-expressed with Bx biosynthetic genes. MYB31 protein activation of several Bx biosynthetic gene promoters was confirmed using a dual fluorescence assay. Using gene silencing, we found that a significant reduction in BXD levels led to increased plant susceptibility to herbivores. TaMYB31 co-expression with target BXD-encoded genes showed a high association with various biotic and environmental conditions. We observed a strong accumulation of these compounds in leaves under drought, salinity, and cold, indicating a yet-known role of BXD in response to abiotic stressors. We also detected a dynamic biosynthesis and accumulation of BXDs depending on leaf age and position, in response to short-term drought, which negatively affects aphid reproduction. This discovery opens new avenues for exploring how a single class of metabolites is involved in adjusting to multiple stressors.

P4-02

## Evolution of cardenolide resistance in *Plutella xylostella*

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The Brassicaceae system is well studied for its co-evolutionary interactions between plants and herbivorous insects. Over a span of 80 million years, these plants evolved diverse mixtures of defensive glucosinolate compounds. In turn, specialized herbivores evolved several coping mechanisms against glucosinolates, reducing the benefits of glucosinolates and requiring new defensive strategies. In an evolutionary recent event, plants from the Brassicaceae genus *Erysimum* gained the ability to produce cardenolides as a novel defence, enabling them to escape some of their herbivores. Therefore, this system provides us with a unique opportunity to study how non-adapted herbivores cope with novel defences, and how resistance to these traits may evolve in the future.

The diamond back moth *Plutella xylostella* is occasionally feeding on *Erysimum* despite being partially susceptible to cardenolides. Making use of its short generation time and rapid adaptation to different insecticides, we generated *P. xylostella* selection lines to accelerate and observe potential adaptations to cardenolides under increased selection pressure. We reared eight replicate populations of *P. xylostella* on *Erysimum* plants for 25 generations and contrasted them to eight control populations reared on Broccoli plants. We measured phenotypic traits of individuals in each selection line by measuring larval host preference, growth rate, and life history changes at regular intervals throughout the experiment. After 25 generations of selection, performance of *Erysimum*-evolved lines remained lower on *Erysimum* plants than on Broccoli plants, but was slightly yet significantly improved relative to Broccoli-evolved lines. While the magnitude of evolutionary responses may thus have been limited by genetic diversity in the ancestral population, our results nonetheless demonstrate the potential for *P. xylostella* to adapt to new host and their toxins. Genotyping of evolved lines and their ancestors will further demonstrate to what extent populations underwent parallel change in response to the *Erysimum* selection regime.

P4-03

**Intra-plant variation in foliar defense and nutrition of *Erysimum cheiranthoides* shape herbivory by *Plutella xylostella***Kunqi Wang, Tobias Zuest*University of Zurich, Zurich, Switzerland*

Plants invest a substantial fraction of their resources into defence against herbivores. However, as defences are often costly to produce, their allocation within a plant can vary substantially, with only the most valuable tissues being best defended. In an evolutionary recent event, plants in the genus *Erysimum* (Brassicaceae) evolved the ability to produce cardenolides, a new class of chemical defence that is co-expressed with ancestrally conserved glucosinolates (GSL). While the potent new defence has allowed *Erysimum* to escape many of its herbivores, the dual investment likely imposes a substantial drain on the plant's resources, making optimal intra-plant allocation essential. Larvae of the GSL-resistant diamondback moth *Plutella xylostella* continue to feed on *Erysimum cheiranthoides*, but when given a choice, appear to avoid the uppermost, youngest leaves on the plant. In accordance with optimal defence theory (ODT), we found that younger leaves had higher concentrations of cardenolides, but glucosinolates and several other traits also varied with leaf age. We therefore performed a set of increasingly mechanistic choice and performance assays to identify the traits involved in the behavioural response of *P. xylostella* larvae. While we found consistent avoidance (antixenosis) of the uppermost leaves for whole plants, detached leaf discs, and even with cardenolide-containing leaf extracts, performance under no-choice conditions (antibiosis) was only affected on whole plants and leaves. Therefore, even though intra-plant variation in cardenolides likely plays a role in directing *P. xylostella* away from the most valuable plant tissues, cardenolides surprisingly do not appear to present a universal defence against this highly versatile herbivore.

P4-04

**Plant- and plot-level intraspecific chemodiversity shape herbivore, pollinator and predatory arthropod communities.**Lina Ojeda-Prieto, Eliecer L. Moreno, Robin Heinen, Wolfgang W. Weisser*Technical University of Munich, Munich, Germany*

The consequences of intraspecific chemodiversity for interactions between individual plants and their interaction partners receive increased attention. However, how variation in chemical diversity in groups of plants shapes arthropod communities is not fully understood. Here, we designed a field experiment with 84 plots with six plants each differing in the number and composition of *Tanacetum vulgare* chemotypes present in a plot, creating a range of chemotype richness (i.e., 1, 2, 3 or 6 chemotypes per plot). Over three summer seasons and 24 time points, we observed the occurrence and abundance of herbivorous, pollinating and predatory insect communities at the plot level. While chemotype richness did not strongly affect the occurrence of any group, it negatively affected abundance of herbivores, driven by strong effects on a dominant specialized aphid, *Uroleucon tanaceti*. In contrast, chemotype richness positively affected pollinator abundance, while predator abundance remained unaffected. We then assessed whether presence of each specific chemotype in a plot affected occurrence and abundance patterns and found that all chemotypes affected arthropods in some way. In the first year, chemotypes tended to have a positive effect on herbivores, which shifted to mixed effects in subsequent years, indicating that plant chemistry may serve as an attractant or repellent. Pollinator communities exhibited order-specific positive responses to chemotypes, indicating an important role for chemistry as a cue for host finding in pollinators. Predator communities were only weakly affected by chemotypes, and the effects were taxon- and chemotype-specific. Our results suggest that chemodiversity is a strong partial driver of herbivore and pollinator communities. How chemodiversity affects arthropod-mediated ecosystem processes, including pollination and herbivore control will be an important next step to understand the importance of intraspecific chemodiversity in ecosystem functioning.

P4-05

**Herbivore diversity induces phytochemical diversity, with implications for a subsequent herbivore**

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Plant secondary metabolites are key in plant insect interactions, and the diversity of phytochemicals in plants has been demonstrated to play important ecological roles at various spatial and temporal scales. Here, we focus on the phytochemical diversity that follows induction by insect herbivores. Considering the specificity that characterizes plant responses, we hypothesized that increasing herbivore diversity results in the induction of a greater diversity of metabolites in plants. To test this hypothesis conceptually, we employed a "dual herbivory" experiment on cabbage plants that we inoculated with generalist aphids and moth caterpillars, while standardizing total herbivory loads according to a preceding calibration experiment. The results confirm an increase in general phytochemical richness under combined herbivory compared to herbivory by each herbivore species alone. Furthermore, whereas caterpillar herbivory resulted in a strong reduction in metabolite Simpson diversity, representing reduced evenness in metabolite abundances, Simpson diversity values were as high under combined herbivory as in uninduced plants, despite the presence of caterpillars. We additionally conducted a field survey in commercial lettuce plots spanning various plant varieties and agricultural practices. We found a positive association between herbivore functional diversity and phytochemical diversity, lending additional support to the induced diversity hypothesis at the herbivore community level.

Building on these results, we studied the effects of induced alpha and beta phytochemical diversity on the behavior and performance of a subsequent herbivore. Following plant induction by topical administration of SsA, Me-JA, or both, we offered pairs of leaf discs to caterpillars in feeding choice experiments. We found that induced alpha phytochemical diversity is detrimental to larval growth and survival, consistent with the metabolite screening/synergy hypotheses. We also found that under induced beta phytochemical diversity, caterpillars perform frequent diet mixing and achieve greater performance than those subjected to non-choice diets of any type of plant induction. Taken together, these results suggest reciprocal relationships between herbivore interaction diversity and the phytochemical landscape at the level of individual plants and plant patches, implying a potential feedback loop between these states over short-term temporal scales.

P4-06

## **Plant-insect-symbiont interaction networks along elevational gradients**

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Elevational gradients are a useful approach to assess how abiotic conditions shape terrestrial communities, and to forecast how these communities will be impacted by future climatic conditions. Plant and animal diversity usually decrease with elevation, but how elevation alters species interactions and rewires ecological networks is far less understood. With elevation environmental uncertainty increases while resource competition is relaxed, leading to the evolution of a broader diet breadth and to networks that are more intertwined or connected. These effects, however, vary depending on the guild of species studied and whether networks are mutualistic or antagonistic. In this study we sampled thrips (a group of minute insects) on plants along replicated elevational gradients. We built quantitative networks of insects thriving on plants, and of bacteria colonising insects. Network analyses revealed that, in agreement with our hypothesis, networks were dominated by generalists at high elevations, but this effect varied between the plant-insect and the insect-microbe networks. I will discuss the importance of our network approach to understand the resilience of herbivores to environmental disturbances, and the dual nature of insect-associated bacteria as either pathogens or mutualistic symbionts.

P4-07

### Variation in virulence of thrips species and populations on wild *Chrysanthemum*

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Populations of pest insects can differ in their responses to resistant plants, which can significantly impact the durability of plant resistance. Differential fitness of biotypes or populations of thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) has been reported previously and, furthermore, new invasive thrips species, such as *Thrips parvispinus* Karny (Thysanoptera: Thripidae), may not be affected by the resistance. In this study, we assessed the virulence of *F. occidentalis* populations collected in the Netherlands on various *Chrysanthemum* accessions and characterized the genetic diversity of these populations. We also examined the resistance of the *Chrysanthemum* accessions against *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and *Thrips parvispinus*. Significant differences in the development of thrips larvae (from the L1 to L2 stage) across five *F. occidentalis* populations on the five evaluated *Chrysanthemum* accessions were found. Two accessions, *Chrysanthemum seticuspe* PB-MB133 and Penny Lane, were consistently resistant, exhibiting low larval development for all *F. occidentalis* populations. Mitochondrial CO1 gene analysis revealed five distinct haplotypes among *F. occidentalis* individuals from different populations, belonging to both the glasshouse and lupin strains. Furthermore, when comparing thrips larval performance on various *Chrysanthemum* accessions for the three thrips species, *F. occidentalis*, *T. tabaci*, and *T. parvispinus*, we found significant effects of plant accession, thrips species, and their interactions on larval development. Penny Lane exhibited suppression of larval development for only *F. occidentalis*, whereas *C. seticuspe* PB-MB133 suppressed larval development for all three thrips species tested. Interestingly, *C. seticuspe* PB-MB132, previously identified as susceptible to *F. occidentalis*, suppressed *T. parvispinus* development, indicating that in *C. seticuspe* multiple mechanisms of resistance might be present. In conclusion, our findings demonstrate that thrips populations infesting *Chrysanthemum* differ in virulence, and they highlight the importance of screening with multiple populations. Moreover, our study identified *Chrysanthemum* accessions exhibiting resistance against multiple thrips species.



P4-08

## **Reciprocal impacts between herbivory and phenology in common evening primrose (*Oenothera biennis*)**

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Reproductive phenology is the seasonal timing of events associated with reproduction, in plants, this includes the timing and duration of flower and seed production. Herbivory has been shown to induce plastic shifts in plant reproductive phenology in a multitude of studies, but varying substantially in magnitude and direction. Here we investigate the cause and consequences of herbivory-induced phenological shifts. Specifically, we ask 1) what aspects of herbivory (resource loss or hormonal signaling) induce phenological shifts? and 2) how do phenological shifts mediate temporal co-occurrence of plants and seed predators and ultimately affect plant fitness? To answer these questions, we grew many genotypes of *Oenothera biennis* (Onagraceae) in a common garden in Central NY. We used jasmonic acid (JA) to test the effects of hormonal signaling and 50% leaf removal to test resource loss. Results show that resource loss had a genotype-dependent effect on time to first flower, while natural herbivores induced delays and altered total floral distribution. Using structural equation modeling, we show that JA, resource loss, and natural herbivory had direct effects on seed predator damage and plant fitness, as well as indirect effects mediated by phenology. Herbivory-induced shifts in phenology were correlated with decreases in seed predator damage. These results highlight phenological shifts as an important mechanism mediating the outcome of sequential herbivory in plant-insect interactions.

P4-09

**Being first: The preference for volatiles from plants infested with small and young aphid colonies can help aphid parasitoids to avoid intraguild predation**

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One key factor that determines the fitness of endoparasitoids is the survival of their larvae that feed inside the insect hosts. Parasitoid larvae face a high risk of intraguild predation if their host is attacked by a predator. Aphid predators are known to accumulate in large aphid colonies and aphid colonies often last only one to two weeks in the field before predators eradicate them. Aphid parasitoids may try to avoid this fate of intraguild predation for their larvae by selecting young and small aphid colonies for oviposition. Indeed, we found in an olfactometer experiment that the aphid parasitoid *Diaeretiella rapae* was most strongly attracted to volatiles emitted by plants infested for just one day with 25 aphids. Volatiles from plants that were infested for more than one day or with 100 aphids were less attractive. *D. rapae* was even attracted to volatiles from plants that had been infested with just one adult aphid for one day. The ability of parasitoid females to detect very small and young aphid colonies and even individual adult aphids may provide their larvae with enough time to finish larval development before predators detect the growing colony.

By using the red and green color morph of the pea aphid, we were able to show that survival of individual parasitized aphids (one color morph) and unparasitized aphids (other color morph) and mummification rate in the presence of predators was highest in small aphid colonies, as these received less hoverfly eggs in cage and semi-field experiments.

P4-10

### **It's all about balance: Trade-off between growth and defence against insects in *Brassica nigra***

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In order to maximize fitness, plants have to balance growth and defence in a way that allows them to outcompete neighbouring plants. It is commonly believed that the metabolic cost of defence results in a decrease of energy available for growth and reproduction. This concept is referred to as the growth-defence trade-off. In addition to the resource trade-off, regulation of growth and defence are physiologically linked and a response in growth or defence may have ecological costs as well. Ecological costs of defence are the costs related to complex interactions between plants, herbivores, natural enemies of those herbivores and pollinators. Defence responses in a plant could lead to attraction of other herbivores or change the interaction between the plant and pollinators. Here, we explored the effect of induced plant growth under simulated plant-plant competition on herbivore performance and feeding position on the plant. Plants were exposed to light with a low red to far-red ratio (R:FR), resulting in a shade avoidance response. These plants showed increased growth and a decreased number of leaves and leaf angle compared to plants under a daylight spectrum. The plants were challenged with two rounds of consecutive herbivore feeding, either by *Pieris napi* caterpillars or *Brevicoryne brassicae* aphids. Resistance of plants to herbivore attack was dependent on an interaction between the light conditions and the order and type of herbivore attack. Moreover, these conditions affected the feeding position of herbivores in the plant canopy. This research shows that growth in response to plant-plant competition intricately affects resistance of plants to dual herbivore attack. My future research will show if these differently induced plant phenotypes have an effect on plant fitness and the entire insect community around the plant under field conditions.

P4-11

## Ecological factors outweigh plant physiology in predicting plant defence to dual herbivore attack: A meta-analysis

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Plants often deal with multiple herbivores during their lifetime. Herbivores may arrive at different moments in time, attack different plant organs, vary in their feeding style and fitness impact on the plant. Therefore, plants have a set of constitutively expressed and inducible defence traits to mitigate the impact of herbivory. Inducible defences are activated upon herbivory, and the signalling pathways that regulate the defence response depend on the identity of the attacker. The interactions among signalling pathways ensure that plants can tailor its response to specific herbivores. However, it is challenging to predict plant resistance against multiple herbivores. Physiological hypotheses involving antagonism between jasmonic and salicylic acid defence signalling pathways cannot fully explain plant resistance against multi-herbivory and recent papers pose alternative ecological hypotheses. These alternative hypotheses include the importance of the tissue attacked, plant life history and the likeliness to encounter specific combinations of herbivores. To explore multiple ecological hypotheses, we performed a meta-analysis assessing the outcome of dual herbivory compared to single herbivore attack. Specifically, we assessed whether plant identity and various herbivore traits affected subsequent response types such as herbivore performance, preference and plant performance. Our analysis identifies that subsequent herbivores do not show preference for induced or clean plants, but generally performed better on clean plants, while plant performance was similar after single or dual herbivory. Moreover, the outcomes of dual herbivory could not be predicted by hypothesis of antagonism among signal transduction pathways. The importance of ecological moderators differed per response type and result in contrasting outcomes for herbivore performance, preference and plant growth. Besides, the overrepresentation of economically important plants and herbivore species in research on dual herbivore attack may conceal species specific effects and evolutionary shifts in plant defence strategies. Overall, we highlight the importance of the measured response on conclusions about dual herbivory and explore which ecological factors are most important in determining plant resistance to dual herbivore attack.

P4-12

## **Have metabolomics methods shifted evidence for the Latitudinal Biotic Interactions Hypothesis for plant chemical defense? A meta-analysis**

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### Background

The Latitudinal Biotic Interactions Hypothesis posits that investment in traits which mediate species interactions, such as plant chemical defense against herbivores, should increase towards the equator in alignment with species density and diversity gradients. Empirical evidence for this hypothesis as it relates to plant chemical defense has been mixed, and several confounding variables have been suggested to contribute to the inconsistency of results. For example, because there is no established 'standard' method for the quantification or comparison of plant chemicals, there is substantial methodological variation introduced between studies. In this study, we disentangle the potentially confounding impacts of chemical methodology on the relationship between chemical defense and latitude using meta-analysis.

### Methods

We collected papers from Web of Science, PubMed, and JSTOR which quantified chemical defense investment across latitudinal gradients. We calculated effect sizes for each comparison of chemical defense and fit multiple meta-analysis models. We fit a model including all effect sizes and then ran subsequent models with potentially confounding chemical factors as moderators, including constitutive vs. induced (model 2) and direct vs. indirect (model 3) defense strategies, compound classes (e.g., phenolics, saponins, terpenoids, alkaloids) (model 4), diversity vs. abundance chemical metrics (model 5), and untargeted vs. targeted metabolomics methods (model 6). We tested additional moderators relevant to plants (e.g., growth form, native range, tissue source) to assess alternate sources of heterogeneity among studies.

### Results

Preliminary analysis of 10 papers and 37 chemical defense measurements suggests an overall positive relationship between chemical defense investment and latitude using a fixed effects model. However, Q-test and I<sup>2</sup> values reveal substantial heterogeneity, indicating a better fit of a random effects model which is negative but non-significant. As yet, none of the moderators we tested are able to explain the heterogeneity observed, suggesting that sources of variation other than chemical methodology may impact the relationship between chemical defense and latitude. Alternatively, the heterogeneity may be an artifact of a small sample size. Future work will expand our dataset and test additional moderators, elucidating the role of chemical methodology in understanding latitudinal patterns in plant chemical defense.

P4-13

### The impact of landscape and weather variables on the seasonal arrival and population abundance of the green peach aphid *Myzus persicae* in sugar beet

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Recent bans on chemical pesticides and limited sustainable alternatives have made pest and disease control highly challenging. Since the European neonicotinoid ban in 2019, sugar beet crops have increasingly suffered from yellowing viruses, leading to significant yield losses. These viruses are exclusively transmitted by aphids, with the green peach aphid *Myzus persicae* serving as the main vector. Knowledge on the population dynamics of *M. persicae* and risk factors is crucial to increase the predictability of aphid infestation and enhance the management of yellowing viruses. In this study, we identified key landscape and weather drivers of *M. persicae* infestation and constructed predictive models to forecast the week of arrival and the extend of infestation in sugar beet. For this, we first related the cumulative and maximum abundance, and first week of observation of alate and apterous *M. persicae* to a large number of environmental variables, including landscape composition and configuration metrics, and weather metrics calculated over different critical time windows. Next, generalized linear models were used to analyze the response of *M. persicae* to combinations of selected variables in three categories: (i) landscape drivers, (ii) weather drivers, and (iii) combined drivers. Higher winter temperatures, wind direction and early sowing favored an early arrival of alate and apterous *M. persicae* independently of the surrounding landscape. The abundance of apterous and alate *M. persicae* was favored by higher winter temperatures, with morphotypes showing varying responses to different landscape elements. Arable land, orchards and semi-natural woody landscape were related to the population abundance of alate *M. persicae*, while the population abundance of apterous *M. persicae* was mainly related to arable land, urban landscape and semi-natural herbaceous landscape. We also found that the population of *M. persicae* was not influenced by precipitation or relative humidity. Forecasts that were made using a combination of metrics, supplemented with sowing week and field area, performed better than models that relied solely on weather or landscape predictors. All in all, this study provides evidence that the population dynamics of the green peach aphid in sugar beet is driven by a combination of weather and landscape parameters and can serve as a basis for developing preventive measures in the management of this and other pest populations in open-field crops.

P4-14

## Plant defence plasticity against attack by multiple insect herbivores

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In nature, plants are confronted with a plethora of insect herbivores which have been driving the evolution of plant defences for more than 400 million years. Plants have developed plastic defensive strategies to be resistant or tolerant against their diverse and, sometimes, unpredictable antagonist communities. While our understanding on plant-insect interactions has greatly improved over the past decade, most studies have focused on single or dual herbivore attack. This comes at a cost of not capturing the complexity of interactions occurring in natural ecosystems, in which plants are often interacting – sometimes simultaneously – with multiple insects. In this context, a plant's response to one organism may alter its phenotype to a herbivore-induced one, which in turn influences subsequent or concurrent interactions. Recent studies revealed that the feeding guild, identity, and order of insect herbivores, as well as the timing of the interaction, are all factors that can affect a plant's defence strategy. How this is shaped under situation of attack by multiple insects is largely unexplored.

Our research explores which defence strategies plants may deploy against their antagonistic insect communities, and how this is affected by the specificities of the interaction. We used a wild population of the black mustard *Brassica nigra*, as model plant in our system, and four ecologically relevant insect herbivores: the leaf chewers *Pieris rapae* and *Plutella xylostella*, and the phloem feeders *Myzus persicae* and *Brevicoryne brassicae*. By using an RNA-sequencing approach combined with insect performance, we found that systemic induction by *P. rapae* and *M. persicae*, alone or in combination and at different arrival-time, does not change the transcriptional response of *Brassica nigra* towards subsequent local simultaneous feeders *B. brassicae* and *P. xylostella*. However, the performance of the local stressor *B. brassicae*, but not *P. xylostella*, was altered when other insects were feeding on the systemic tissue. These results suggest that the herbivore-induced plant phenotype does affect subsequent interaction but is not detectable at the transcriptomic level in locally stressed leaves. Additionally, these results indicate an organ-specific canalization strategy, which allow the plant to respond locally to individual attackers.

P4-15

## Plasticity in plant pollination and reproduction in response to insect herbivory

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Environmental conditions such as attack by herbivores can have a large influence on plant reproductive success. The sessile nature of plants creates an additional challenge for reproduction, especially in plants that have a short reproductive window and reproduce exclusively via cross-pollination. *Brassica rapa*, of the cabbage and mustard family, has an annual life-cycle and depends on the visitation of animals for pollination and thus reproduction. It receives cross-pollination from a wide range of pollinators, like honeybees, bumblebees, syrphid flies and butterflies. Like many Brassicaceae, *Brassica rapa* is self-incompatible, meaning that it recognizes and rejects its own pollen.

My research focusses on the effects of leaf and tissue herbivory - by insects such as caterpillars, aphids and beetles – on the pathways of cross- and self pollination in *Brassica rapa*. In the case of cross-pollination, both the quantity and quality of received pollination services may be affected by insect herbivory. For example, insect herbivory can affect the species in the pollinator-community differently. In the case of self-pollination, some degree of plasticity in the level of self-incompatibility could provide the plant with reproductive assurance as relying on cross-pollination only in stressful conditions might prove to be a risky reproductive strategy. To uncover whether there is plasticity in reproductive pathways, we exposed different *Brassica rapa* genotypes to various insect herbivory environments and measured pollination and seed production traits.

I will present the results of a field study that show that manipulated levels of insect herbivory affect seed set and pollinator attraction and community composition in the field. Additionally, seed production traits, as well as pollinator visitation, were found to vary throughout the season. A greenhouse study shows that herbivory can also alter the acceptance of self-pollen. Both studies were performed on different genetic accessions of *Brassica rapa*, revealing that there is a large genetic component in how reproduction is affected by insect herbivory.



P4-16

### **Interplay of soil legacy and plant direct and indirect defenses**

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Growing cover crops between cash crop rotations can significantly improve a wide range of soil properties. Our recent studies suggests that different cover crops, such as pea (leguminous, mycorrhizal), triticale (non-leguminous, mycorrhizal), and radish (non-leguminous, non-mycorrhizal), can alter the constitutive phytochemistry of maize. This observation led us to hypothesize that soil legacy from these cover crops might also shape maize's defense strategies against herbivores, potentially prompting plants to focus on direct defenses in some cases, while in others, indirect defenses are favored. After examining the legacy effects of pea, radish, triticale, and fallow soil on maize defenses against fall armyworm, our findings suggest that cover crop soil legacies influence indirect defenses through predator feeding preferences, evidenced by variations in headspace indole, and direct defenses through benzoxazinoid levels. These results indicate that plants with cover crop exposure might shift toward indirect defense strategies compared to those from fallow soil, potentially due to the different nutrient levels in each soil type.

P4-17

## Using AI and citizen science to comprehend interactive spatial and temporal spread dynamics of invasive herbivorous insects and plant diseases

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Numerous insect species are expanding their natural range boundaries, driven significantly by direct and indirect anthropogenic activities. While non-native herbivorous insects can cause substantial ecological damage to native plant communities, only a few establish themselves long-term. Understanding the reasons behind this success involves examining the abiotic environmental prerequisites and the insects' ability to adapt [1]. No less important, biotic factors are crucial in shaping the ability of range-shifting herbivorous insects to establish and spread. While the availability of host plants has been extensively studied [2], these insects are rarely alone in their journey. Plant diseases caused by pathogenic fungi have also been rapidly expanding. The mutual influence of global insect and pathogenic fungal spread remains unclear. Comprehensive datasets on plants, insects, and fungi are essential to address this question. However, distribution data for insects and especially pathogenic fungi are often lacking.

Citizen science programs provide a robust database of plant photographs that could reveal both infestations with insects and pathogenic fungi. This data could form a solid foundation for information on species' distribution over space and time. Convolutional Neural Networks (CNN) have become invaluable in analyzing ecological image data, identifying plant species, plant functional traits [3], and mapping forest dieback in aerial imagery [4]. We are currently developing an algorithm for this purpose, expecting CNNs to identify leaves infected by insects and pathogens at a human level. This technique will help us to efficiently analyze the interactive spatial and temporal spread dynamics of invasive herbivorous insects and plant diseases.

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P5-01

## Whitefly (*Bemisia tabaci*) resistance in pepper (*Capsicum* spp.) and its association with trichomes

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Pepper (*Capsicum* spp.) is a strategic horticultural crop for both fresh and processed products. However, its cultivation faces challenges, with one major threat being the whitefly, *Bemisia tabaci* (Genn). To date, control of whiteflies relies on chemical pesticides, but this poses environmental risks. Therefore, the use of pepper cultivars resistant to whiteflies is a good alternative to promote sustainable agriculture. To achieve this, the identification of whitefly resistance sources and the study of the resistance mechanisms are important. In this study, we searched for *Capsicum* accessions resistant to *B. tabaci* MEAM1. Forty pepper accessions underwent screening through a no-choice clip cage assay, evaluating the adult survival and oviposition of female adult whiteflies. One accession (CA9) with high mortality of whiteflies and low oviposition was found. Next, we confirmed the resistance of CA9 accessions through a population-building experiment assessing the number of nymphs, pupae, and adults. We found that this accession allowed for a low population buildup. Previously, resistance to whiteflies was associated with the presence of glandular trichomes on the leaves. Therefore, we investigate trichome presence and density in CA9 and susceptible *Capsicum* accessions. On CA9, we identified an abundance of glandular trichome type VI. Moreover, the glandular trichome type VI showed a negative correlation with the number of adults, eggs, nymphs, and pupae. Thus, we conclude that CA9 accession could serve as a source for whitefly resistance, with a possible role for type VI glandular trichome as a resistance mechanism. Future studies of the genetics and mechanism of this resistance should be done to confirm that glandular trichome type VI is responsible for whitefly resistance in *Capsicum annuum*.

P5-02

### **Molecular and behavioral aspects of sweet taste perception in the phloem-feeding whitefly *Bemisia tabaci***

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Sweet taste sensation is crucial for feeding site allocation, diet assessment, and appetite regulation in insects. Ability to successfully conduct these three tasks is extremely important in phloem-feeding, as the feeding site is buried deeply inside the leaf and is highly rich in sugars (mainly sucrose) up to concentrations that create osmoregulation challenges. The molecular mechanism of sweet taste perception is based on the activity of gustatory receptors that are dedicated to the detecting of sugars. Using complementing bioinformatics tools, we identified in a previous work four sweet taste gustatory receptors in the phloem-feeding whitefly *B. tabaci* (BtabGR1-4). We also brought evidence that *B. tabaci* Gustatory receptor 1 (BtabGR1) is highly tuned to sucrose detection and demonstrated, using artificial diet assays, its putative role in feeding-site location. Here, we present further research on BtabGR1 using in-planta and inhibition assays. Also, we present the screening results for determining the receptive range of the three other sweet receptors of *B. tabaci*, BtabGR2, BtabGR3 and BtabGR4. Based on our current knowledge, we make an attempt to provide an initial framework for explaining how sugar perception works in phloem-feeding insects. We focus mainly on three feeding activities: locating a feeding site, accepting it and regulating sugar intake.

P5-03

## Aphid resistance in pumpkin and squash, uncovering potential resistant sources

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Pumpkin and Squash belong to the genus *Cucurbita* that includes five cultivated species that are grown worldwide. However, the production of these economically important crops is threatened by aphids. *Aphis gossypii*, a phloem feeding insect, is one of the major species of aphid causing considerable damage to these crops, through direct damage and transfer of viruses. Management of aphids is still largely done using chemicals. As an alternative, resistant varieties could be used. However, this requires sources of resistance, which have not been found so far. To identify resistance sources to *A. gossypii*, we screened a total of 448 accessions, consisting of four cultivated species (*C. argyrosperma*, *C. maxima*, *C. moschata* and *C. pepo*), and two wild relatives, (*C. ecuadorensis* and *C. lundelliana*), from two genebanks. These accessions were screened in no-choice, clip-cage assays for resistance to *A. gossypii*. By assessing survival and reproduction of this insect, we identified 44 potentially resistant accessions. Some of these selected accessions were crossed with susceptible accessions to generate eight F1 populations that were further phenotyped for aphid resistance. This phenotyping resulted in accessions segregating for aphid resistance. In the future, generation of F2 mapping populations for QTL analysis and characterization of resistance mechanisms will be performed to further study the resistance in *Cucurbita* against aphids. In this way, this research will contribute towards the development of resistant *Cucurbita* varieties.

Keywords: *Cucurbita*, Aphid, phloem feeder, Host plant resistance, Resistance mechanism, and QTL

P5-04

## **Reducing aphids on barley by exploiting beneficial species interactions with rhizobacteria and natural enemies**

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Aphids reduce agricultural yield and quality by extracting plant nutrients and vectoring diseases. Pesticides, although effective, can damage ecosystems and pest populations are evolving resistance. There is increasing evidence for using soil microbiome manipulation as a pest control strategy. We inoculate barley with beneficial rhizobacteria to reduce aphids by inducing plant defences and recruiting natural enemies such as parasitoid wasps in the field. We present results from full season outdoor pot and glasshouse experiments using rhizobacteria inoculation across different barley varieties to explore aphid and natural enemy colonisation. We saw earlier arrival and more parasitoid wasps on rhizobacteria inoculated barley compared to the control treatment. Aphid suppression was variable between barley varieties and across time, with some inoculated plants even attracting more aphids in the outdoor pot experiment. We investigated this further through controlled aphid choice experiments and chemical analysis of barley leaves. Ongoing work explores effects of rhizobacteria inoculation on plant volatiles in relation to parasitoid behaviour as well as aphid susceptibility to parasitism. Overall, we aim to provide practical solutions for agriculture and food security.

P5-05

## The role of protective symbionts in mediating aphid interactions with beneficial rhizobacteria and parasitoids on barley

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Aphid pests are increasingly being managed via biological control agents, such as parasitoid wasps or entomopathogenic fungi. In addition, rhizobacteria inoculation of plants to boost plant defences against insect pests also has strong potential for sustainable agriculture. However, aphids host facultative bacterial endosymbionts that can provide protection from natural enemies, reducing biocontrol effectiveness, with unknown effects in response to rhizobacteria-inoculation of host plants. Protective symbionts often impose a fecundity cost to the aphid, leading to cost-benefit trade-offs and variation in symbiont infection rates within aphid populations. We present data on aphid symbiont prevalence from a field trial where we inoculated barley plants (*Hordeum vulgare*) with two different rhizobacteria (*Acidovorax radialis* and *Bacillus subtilis*). We also present data from preliminary experiments using field-collected aphid lines (*Sitobion avenae*) with different symbionts, that vary in population growth and susceptibility to parasitoids. Future work will create experimental aphid lines differing in symbiont infection status using a microinjection protocol for artificial symbiont transmission. This project aims to shed more light on the role of aphid protective symbionts in a community wide context and inform the development of effective biocontrol strategies.

P5-06

**Revealing the dynamics of defense strategies in *Setaria* plants across developmental stages under aphid infestation**Anmol Dayashankar Mishra, Yoshiahu Goldstein, Vered Tzin*Ben-Gurion University of the Negev, Be'er Sheva, Israel*

Plants have evolved multiple defense strategies to resist or avoid insect damage, including the production of toxic chemical compounds, the formation of physical barriers, and metabolic adjustments through the reallocation of essential compounds. As plants progress through their growth phases, the allocation of resources between development and defense may shift, leading to variations in defense responses at different developmental stages. However, the impact of these defense strategies along various developmental stages, has not been fully explored. To understand the dynamic trade-off between growth and defense, the monocot plant, *Setaria viridis*, was infested with *Rhopalosiphum padi* aphid, one of the most economically significant pests worldwide, known for causing substantial crop yield losses. We conducted a transcriptome analysis of *Setaria* grown from the 3rd-week young seedlings (vegetative stage) to the 7th-week (reproductive stage), infested with *R. padi* aphid. We identified the induction of both serotonin and phenylpropanoid pathway-related genes between the 3rd-7th weeks. These pathways are crucial for synthesizing various secondary metabolites that aid in plant defense against herbivory. Total chlorophyll content was reduced in the 3rd and 4th weeks, however, downregulation of photosynthesis-related genes was observed not only in these weeks but also in the 6th week. Moreover, *S. viridis* showed significant induction of trichomes, limited to the 3rd to 5th weeks, in response to aphid infestation. The results deepen our understanding the changes of growth and defense trade-off, by revealing which growth phases are more vulnerable to insect infestation due to weaker chemical and physical defense mechanisms. This knowledge aids in the development of sustainable pest management strategies.



P5-07

## Testing The Optimal Defense Theory in indirect plant defenses mediated by ant-hemipteran interactions

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The Optimal Defense Theory (ODT) postulates that, reproductive structures should be more defended because they contribute most to a plant's fitness and have the highest probability of being attacked by herbivores. Ants can provide indirect defense to plants through their mutualistic interactions with hemipteran insects. In this well-studied interaction, ants provide protection to hemipterans against their natural enemies (e.g., predators and parasitic wasps) in exchange for the sugar-rich honeydew secreted by hemipterans. In turn, ants farming hemipterans can indirectly benefit plants through suppression of other harmful herbivores. Despite the numerous investigations of this food-for-protection mutualism, patterns of optimal allocation to indirect plant defenses mediated by ant-hemipteran interactions have not been investigated previously. In this study, we investigated whether allocation to indirect plant defenses mediated by ant-hemipteran interactions and the effectiveness of such indirect defenses differ between vegetative branches (with only leaves) and reproductive branches (with leaves and inflorescences) of the tropical shrub *Solanum lycocarpum*. For this, we selected plants with and without aggregations of the ant-tended hemipteran *Enchenopa brasiliensis* in both reproductive and vegetative branches. We then estimated indirect defenses (plant resources for ants and ant attraction), and their effectiveness in terms of plant protection by ants (i.e., damage and survival of leaf-chewing herbivores). Supporting ODT predictions, we found that the sugar concentration in plant sap, and consequently in hemipteran honeydew, was higher in reproductive than in vegetative branches. However, this increase in honeydew quality did not result in increased ant attraction to reproductive branches. Additionally, contrary to ODT predictions, we found that ants attending hemipterans did not provide effective plant protection against leaf-chewing herbivores, whether feeding on reproductive or vegetative branches. In particular, we found that herbivore leaf damage and survival were greater in plants with ant-hemipteran interactions compared to those without. This increase in leaf herbivory was consistent in both reproductive and vegetative branches. Overall, our study demonstrated that the patterns of allocation to indirect defenses mediated by ant-hemipteran interactions in *S. lycocarpum* plants did not support the ODT predictions.

P6-01

## Do sequestered glucosinolates protect horseradish flea beetles against entomopathogenic nematodes?

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Phyllotreta flea beetles sequester glucosinolates from their host plants, which are hydrolyzed by endogenous myrosinase enzymes to produce toxic products such as isothiocyanates and nitriles. This so-called glucosinolate-myrosinase system has been shown to protect *P. armoraciae* larvae from a generalist arthropod predator. Here, we investigated whether the glucosinolate-myrosinase system protects *P. armoraciae* larvae and adults from another ecologically relevant group of natural enemies, entomopathogenic nematodes. To determine whether entomopathogenic nematodes are exposed to glucosinolate hydrolysis products when infecting *P. armoraciae*, we quantified the levels of these metabolites in uninjured larvae and adults. Isothiocyanates and nitriles were detected in larvae but not in adults. In larvae, these levels were reduced by approximately 80% after silencing myrosinase gene expression by RNA interference, directly linking myrosinase activity to constitutive glucosinolate hydrolysis in uninjured larvae. We then tested whether infection with the entomopathogenic nematode *Heterorhabditis bacteriophora* activates the glucosinolate-myrosinase system, but found no difference in the level of hydrolysis products in infected and uninfected larvae and adults. Next, we directly exposed *H. bacteriophora* to different concentrations of glucosinolate hydrolysis products in aqueous solutions. At physiological concentrations, these assays resulted in mortality rates of up to 97%, showing that *H. bacteriophora* is susceptible to glucosinolate hydrolysis products. Finally, we tested whether the glucosinolate hydrolysis products present in larvae protect them against entomopathogenic nematodes by exposing control larvae and larvae with silenced myrosinase gene expression to *H. bacteriophora*. Surprisingly, the survival of larvae with high and low myrosinase activity did not differ. This suggests that the levels of glucosinolate hydrolysis products in larvae do not correlate with the ability to survive *H. bacteriophora* attack. To better understand the impact of the glucosinolate-myrosinase system on the interaction between *P. armoraciae* and entomopathogenic nematodes, we currently investigate the effects of glucosinolate hydrolysis products on entomopathogenic nematode-associated bacteria and the impact of the beetle's chemical defense on the population dynamics of entomopathogenic nematodes.

P6-02

## Chemical diversity in hostplants of tenthredinid sawflies

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When phytophagous insects are feeding, they ingest plant secondary metabolites (PSM) that they can exploit as “insect secondary metabolites” (ISM). Here, the aim was to explore a large lineage of insects, the sawfly family Tenthredinidae, and to compare among their species which types of PSM they ingest. Screenings were performed by linking lists of hostplant genera with a dedicated databank of organic compounds. Principal component analyses revealed that among all known hostplants of the tenthredinids, the Ranunculaceae and Lamiaceae are two important plant families because they contain diterpenoids. Within each of the seven known tenthredinid subfamilies, the species were screened and then monophagous species were compared with non-monophagous species (i.e., feeding on one versus more than one plant genus). The chemical diversity of the hostplants was similar between species with a narrow versus larger diet breath in sawfly subfamilies mainly feeding on herbaceous plants, whereas diversity of the PSM was significantly lower for monophagous than non-monophagous species in sawfly subfamilies generally feeding on shrubs and trees. Furthermore, major types of PSM (e.g., glucosinolates, terpenoids, steroid alkaloids, steroid saponins) were those chemicals that are sequestered by tenthredinid larvae to defend themselves against predators. Overall, using one comprehensive dataset about PSM may help to understand several facets of plant-insect relationships, among which the determinants of defensive ISM.

P6-03

## **ABCB transporters and the transport network for sequestered cardenolides**

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Many phytophagous insects not only tolerate and detoxify noxious compounds present in their host plants but even sequester the toxins for their defence. This necessitates specific mechanisms, especially carrier proteins that regulate uptake and transport to specific storage sites or protect sensitive tissues from toxic compounds. We focus on two model systems, the milkweed bug *Oncopeltus fasciatus* and the leaf beetle *Chrysochus auratus*, that both sequester cardenolides from their *Asclepias* or *Apocynum* host plants, and investigate the involvement of ATP-binding cassette subfamily B (ABCB) transporters in this process. In both species several ABCB full transporters could be identified from transcriptomes of adult insects (*O. fasciatus* 4; *C. auratus* 3) that show a pronounced tissue specific expression. Knock-down experiments by RNAi under cardenolide exposure compared to non-exposure identified ABCB transporters involved in the protection of the sensitive nervous tissue but also those responsible for transfer into the defense fluids of *O. fasciatus*. On the other hand, enzyme assays with heterologously expressed transporters were used to analyze the substrate spectra of the different ABCB transporters. Both approaches confirm the crucial role of ABCB transporters for sequestration of cardenolides.

P7-01

## Do options matter? Choice and no-choice assays together reveal chemical mediated resistance of cruciferous plants to the pollen beetle

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The pollen beetle (*Brassicogethes aeneus*) is one of the major insect pests in oilseed rape (*Brassica napus* L., Brassicaceae) causing significant yield losses. As pollen beetles are becoming increasingly resistant to pyrethroids, alternative control strategies are required as part of integrated pest management – such as the breeding of resistant plants. Before performing an intergeneric transfer of resistance against adult pollen beetles into oilseed rape, we have screened a wide range of *Brassica napus* accessions, *B. napus* resyntheses and wild crucifer relatives (Austel et al. 2021). For the screening and the detection of antifeedant kairomones, we have evaluated both a no-choice bioassay on intact plants and a dual-choice assay with detached flower buds under controlled conditions. In the no-choice assay no natural resistance was observed in *B. napus* and its resyntheses, while accessions of *Sinapis alba*, *Eruca sativa* and *Barbarea vulgaris* suffered very little feeding damage. These observations were confirmed by the dual-choice assays in which a flower bud of certain accessions and/or species was offered to a male or female pollen beetle together with a flower bud of our standard oilseed rape cultivar “Express”. However, the dual-choice assay could not detect the finely-tuned differences between resistant and susceptible accessions within a species, such as within *Sinapis alba* or *Eruca sativa*, that were determined in no-choice assays. Nevertheless, the application of plant extracts or reference substances to the complete main inflorescence was not feasible with the no-choice assay. The dual-choice assay with single flower buds and the application of crude plant extracts or standard compounds very well revealed the effect of secondary plant compounds on the feeding behaviour of the pollen beetle. While flavonoids (aglycons as well as their glucosides) did not show any deterrent effect, certain aromatic glucosinolates showed strong deterring effects on feeding pollen beetles. In conclusion, both choice and no-choice assays are needed to obtain a full view of the host plant - insect interaction and its mediation by plant compounds.

P7-02

## Variation in natural resistance and attraction of the cabbage root fly (*Delia radicum*) in *Brassica napus* lines

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Rapeseed (*Brassica napus*) is a widespread crop in Europe used for fuel production, human consumption and livestock farming. With 11.6 million tons per year, Europe is one of the main producers worldwide (fao.org/faostat). In recent decades, more and more synthetic chemical pesticides have been banned due to their negative impacts on human health and ecosystems. This requires the development of sustainable and environmentally friendly alternatives for pest control. Pests also include belowground herbivores, although these are often not considered. A common belowground pest in *B. napus* is the cabbage root fly *Delia radicum*. The larvae live belowground and burrow into the taproot, where their feeding damage affects crop yields. In this project, we focus on the natural resistance of *B. napus* lines on *D. radicum*. While the larvae cause damage to the roots, the adults do not harm the plant. However, the females choose the host plant for the larvae by selecting it for oviposition. We focus on both life stages and study whether *B. napus* lines differ in susceptibility to *D. radicum* larvae. Furthermore, we want to know whether the attraction of females differs among *B. napus* lines. Although we found no correlation between plant-produced volatiles or leaf wax metabolites and oviposition, we indeed detected differences in oviposition preference and larval damage between *B. napus* lines. Interestingly, larvae and females prefer and dislike the same plant lines. Finding natural traits for the resistance to *D. radicum* larvae and the attraction of females is a first step towards the development of sustainable and environmentally friendly oilseed rape production.

P7-03

**Opposite effects of herbivory-induced rhizosphere changes on plant-caterpillar interactions in wild and cultivate cabbage plants**Kris De Kreek, Rieta Gols, Karen Kloth, Marcel Dicke*Wageningen University & Research, Wageningen, Netherlands*

Plants can reduce insect-herbivore feeding damage by increasing direct plant defence or recruiting natural enemies of the herbivores. Additionally, recruitment of soil microorganisms may prime plant defences in both below- and aboveground plant tissues. We hypothesise that plants may recruit such microorganisms to the roots through a belowground 'cry for help', leading to a change in the rhizosphere microbiome. Whether these recruited microorganisms help plants in their defence against herbivorous insects remains to be assessed. Here, we investigated whether cabbage plants enhance their defences against insects when growing on soil previously conditioned by plants infested by insects. We did this through rhizosphere transfer in plant-soil feedback (PSF) experiments involving four caterpillar and three aphid species, two cabbage accessions (wild and cultivated *Brassica oleracea*), and different soil types. Insect performance was assessed over time and defence gene expression was measured in the first two hours of insect feeding. Insect-herbivory-induced soil conditioning affected the performance of the caterpillar *Mamestra brassicae*. On wild cabbage, insect-induced PSF had a neutral to negative effect on *M. brassicae* performance and this effect became more pronounced over time. In contrast, insect-induced PSF was neutral to positive on *M. brassicae* on cultivated cabbage and did not differ between soil types. Insect-herbivory-conditioned soil negatively primed jasmonic acid (JA) responsive genes in cultivated cabbage, resulting in attenuated upregulation of the JA pathway upon *M. brassicae* feeding. This may explain the positive effect of insect-herbivory-induced soil conditioning on *M. brassicae* performance. Thus, changes in cabbage rhizosphere upon insect attack may negatively affect plant defence and increase herbivore pest pressure in cultivated cabbage while the opposite occurs in its wild relative. This research gives more insight into the role of the soil microbiome in plant defence against insects.

P7-04

## Strip intercropping increases predation and parasitism of eggs and larvae of key agricultural pests on cabbage

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Simple agro-ecosystems are inhospitable habitats for beneficial insects, hampering the provision of ecosystem services like biocontrol. Higher crop diversity in the field is hypothesized to increase the abundance and activity of naturally occurring beneficial insects by providing alternative prey or hosts, nectar, and favorable microclimate. We investigated the impact of crop diversity on biocontrol efficacy through a field experiment conducted over the entire growing season, employing three different crop designs in two locations in the Netherlands. A large monoculture of cabbage was used as a reference for a simple agro-ecosystem, while strip cropping fields featuring either two (cabbage paired with oat) or six different crops (cabbage, oat, potato, grass-clover, pumpkin and faba beans) were established to observe the effects of crop diversity on biocontrol. In all crop designs, we focused on predation and parasitism of pests on the cabbage crop. Egg predation and parasitism were measured using sentinel eggs of *Mamestra brassicae*. Furthermore, a release and recapture method was used to assess larval parasitism of *Pieris brassicae* caterpillars. Strip intercropping significantly increased predation and parasitism rates of *Mamestra brassicae* eggs, as well as the parasitism of *Pieris brassicae* larvae. Intercropping with six crops enhanced egg parasitism compared to strip intercropping with two crops. However, no difference was found in egg predation and larval parasitism between the two strip intercropping systems. The increase in larval parasitism in more diversified cropping systems was consistent in both locations. Interestingly, strip intercropping increased egg predation in one location and egg parasitism in the other, indicating a potential complementary effect. These results underscore the potential of crop diversification as a viable strategy for enhancing biocontrol in agricultural settings. By providing a more heterogeneous environment with varied resources, diverse agro-ecosystems can support natural enemies, thereby promoting more effective pest management practices.



P7-05

## **Strip cropping enhances insect biodiversity and natural pest control.**

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There is increasing interest in agricultural production systems that are biodiversity-friendly and have a reduced dependency on agrochemicals. Strip cropping, where multiple crops are grown in narrow strips in the same field, may support a higher diversity of beneficial organisms, but the evidence for this is still scant. Over the course of four years, we have studied whether strip cropping can enhance beneficial trophic interactions and biodiversity. We found that strip cropped fields had on average 15% more ground beetle species than monocultural fields. Furthermore, strip cropping enhanced parasitism rates by parasitic wasps and reduced the survival of herbivores. Yet surprisingly, we found higher oviposition of the cabbage root fly on cabbages in strip cropping, although we did not observe differences in the number of larvae later in the season. This indicates that survival of root fly immatures is lower in strip cropping, and that egg numbers might not be good predictors of crop damage in more diverse cropping system. Similarly, we observed that the effect that aboveground herbivores have on crop production differed among cropping strategies. This indicates that economic damage thresholds derived for monocultures are not representative for more diverse cropping systems.

Simultaneously, strip cropping is steadily being taken up, with over 60 farmers currently practicing strip cropping throughout the Netherlands. Therefore, the time is ripe to step away from controlled experimental fields and to quantify the effectiveness of strip cropping as a pest management tool in practice. This summer, we will visit around 20 farms that are growing cereals and/or potatoes in strip cropping. At these farms, we will measure the abundances of slugs, aphids and Colorado potato beetles, and their ground- and canopy-dwelling natural enemies. Besides discussing the above results from previous research, I hope to show a glimpse of the first results from this diversity of strip cropping farms during SIP 2024.

P7-06

**A multi-experimental approach to study broad mite (*Polyphagotarsonemus latus*) resistance in jute mallow (*Corchorus* spp.)**

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In this study, we investigated broad mite (*Polyphagotarsonemus latus*) resistance in 300 jute mallow (*Corchorus olitorius* & *Corchorus capsularis*) accessions (collected in Africa and Asia) combining three-year experiments of injury assessments in field and greenhouse assays, population growth, host preference using whiteflies *Bemisia tabaci* as the phoront vector of broad mites, feeding and behaviour assays of both pests using EntoLab, an automated video tracking screening platform of plants for resistance to pests. The analysis of the *P. latus* damage score through time using linear mixed models indicated significant differences among accessions. The damage score for most accessions was consistent over the two years. Furthermore, there was a significant effect of the interaction between accessions and time and of the number of broad mites on the damage score. The damage varied among accessions through time when the numbers of mites were similar, indicating potential resistance of some accessions. Contrary to the field assay results, there was no significant difference in the damage score among accessions in the greenhouse. Damage increased through time on all accessions, and all were heavily damaged within a few weeks after infestation. Differences between the field and greenhouse assays may have been caused by the timing of infestation in the field. Accessions that were damaged late on the field were likely more difficult to be found or less preferred by broad mites and whiteflies. Furthermore, preliminary results of the host preference experiment suggest that some accessions were less preferred by broad mites compared to others. Also, a higher mortality of broad mites was observed on these accessions, suggesting some resistance. Further analysis of the population growth and the feeding and behaviour of mites and whiteflies are underway.

Our results show that combining different experimental approaches can provide a better understanding of the resistance of jute mallow to broad mites and improve the selection of resistant genotypes to include in breeding programs.

P7-07

## Unraveling plant-insect interactions in custard apples: Trade-offs and domestication syndromes

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Exploring plant-insect interactions is key for both evolutionary ecology and practical applications in crop management. The genus *Annona* (Magnoliales: Annonaceae) includes several underutilized and semi-domesticated fruit crops, known as custard apples, and it offers a unique window into plant-insect interactions due to the rich diversity of species and the associated phytophagous insect fauna. Predominantly found in (sub)tropical regions, *Annona* species exhibit a wide array of secondary metabolites that influence insect behavior, yet the intricate dynamics of these interactions remain underexplored. To bridge this gap, we analyzed the phylogenetic relationships within *Annona*, particularly among cultivated species, and the diversity and occurrence of phytophagous insects to identify patterns of insect occurrence. Additionally, using material from a living germplasm collection, we examined the trade-offs and domestication syndromes associated with pest occurrence in the species of *Annona* present in this collection. Our findings indicate that domestication processes, which often select for traits beneficial to human use, may predict pest occurrence in cultivated and wild relatives. Our study underscores the complexity of plant-insect relationships in *Annona*, suggesting significant evolutionary and artificial selection patterns that can provide insights into fundamental ecological principles and pest management.

P7-08

## Understanding the interplay of tomato varieties, *Tuta absoluta*, and natural enemies

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Combining host plant resistance and natural enemies is a promising approach to replace chemical insecticides for the control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), an invasive pest threatening tomato production worldwide. However, morphological resistance traits, secondary metabolites, and plant volatiles of tomato can also affect natural enemies and thus the overall level of pest control. In this study, we aim at characterizing the interaction between *T. absoluta* – tomato varieties – and natural enemies. Therefore, we performed a resistance assessment of 19 tomato varieties including 16 domesticated ones, one wild-domesticated and two wild tomatoes against *T. absoluta*. Tomato varieties affected the oviposition potency, larval mining time, larval development time, leaf area damaged, pupal weight and the emergence of adult *T. absoluta*. Notably, the wild tomatoes *Solanum arcanum* and *S. neorickii* and the domesticated tomato Corona F1 caused detrimental effects on *T. absoluta*. Trichome density did not influence female oviposition but extended larval establishment time on leaflets. Subsequently, six varieties (four domesticated ones and the wild tomatoes *S. arcanum* and *S. neorickii*) that strongly differ in their level of resistance were selected to assess the performance of the egg parasitoid *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) and the larval parasitoid *Necremnus tutae* (Reuter) (Hymenoptera: Eulophidae). Domesticated plants and the wild tomato *S. neorickii* showing lower densities of glandular trichomes had no effect on parasitoid efficacy, in contrast to the wild tomato *S. arcanum* exhibiting the highest density of glandular trichomes and negatively impacted the performance of both parasitoid species. Our findings demonstrate the complex interaction between host plants differing in resistant traits and natural enemies. The impact of plant resistance on these natural enemies as well as implications for the sustainable management of *T. absoluta* are discussed.

P7-09

## Biological control on tomato without type VI trichomes

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Plant glandular trichomes are a target in breeding for enhanced crop resistance. However, predatory arthropods are often hindered by these glandular trichomes in crops such as tomato. The limited number of biological control agents that can be used in tomato makes its biological control system inflexible and, as a result, insufficient upon the emergence of pests that are well-adapted to the trichome-rich environment. What are the opportunities for biological control in tomato that arise when these trichomes are absent? Our project centers around the consequences of taking out the most abundant type VI trichomes for the different players in the tomato biological control system, in the context of building an alternative system for biological control in tomato lacking VI trichomes. To study whether such a tomato variety is more accessible to natural enemies, we investigated the effects of the trichomes on the establishment of generalist predator *Orius laevigatus*. We observed that the success of *O. laevigatus* is indeed related to type VI trichomes: *O. laevigatus* only established in the absence of these trichomes when supplemental food was also provided. This result shows that removing the glandular trichomes from tomato may lead to more opportunities for the use of a wider range of biological control agents in tomato and is worth exploring further.

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P7-10

### **Impact of maize root and shoot damage on predation of *Spodoptera frugiperda* caterpillars by *Polistes* wasps**

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In natural and agricultural ecosystems plants are attacked by herbivores both below and aboveground. Herbivore damage triggers a cascade of physiological and chemical responses in plants, that can influence the behavior of herbivores and their natural enemies, potentially altering the outcome of herbivore-plant interactions. Although numerous studies have explored the effects of individual types of damage on plant responses on herbivore and natural enemy performance, the interactive effects of aboveground and belowground damage remain less understood. We examined how simultaneous above and belowground tissue damage affects the interaction among maize (*Zea mays*) plants, caterpillars (*Spodoptera frugiperda*), and predatory wasps (*Polistes* sp.). In field experiments, maize plants were subjected to different types of mechanical damage: belowground, aboveground and both. Subsequently 2nd instar *S. frugiperda* caterpillars were pinned to a maize leaf and the time of their removal by predatory wasps was determined. Specifically, we tested the hypothesis that combined damage will result in faster caterpillar removal, due to an increase in the chemical responses of plants, likely through the emission of volatiles. We found that simultaneous damage to different plant tissues results in increased predation by *Polistes* wasps, confirming our hypothesis. Additionally, we found that the ratio of volatiles emitted by the different damage treatments was significantly different. Our findings highlight the importance of predatory wasps as potential biological control agents, a role that has often been overlooked and warrants more attention.

P7-11

## The native cinnabar moth - *Tyria jacobaeae*: Is it a suitable biological control agent against the poisonous ragwort species in Germany?

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The rising number of tansy ragwort (*Jacobaea vulgaris* Gaertn.) and related species in grassland in Germany, especially under low management intensity, is highly problematic for their use in hay and silage production due to the poisonous impact of *J. vulgaris* on livestock. In an attempt to evaluate the suitability of the native and specialized cinnabar moth, *Tyria jacobaeae* L. as potential biological control agent, we investigated (1) rearing/maintenance of this obligatory univoltine species (2) effect of controlling *J. vulgaris* by field exposure of reared larvae, as well as their (3) potential of targeting alternative ragwort species. We started out the rearing program in 2022 with field-collected larvae, but noticed soon high death rates, partially due to infection by microsporidia. Based on that, we sampled at 17 different locations in Germany to investigate the natural occurrence of these pathogens in *T. jacobaeae* and found about 50% of the locations bearing infected populations. Hence, we adapted our rearing to exclude any transfer of infected larvae from one location to the other and to work out a protocol for receiving “clean” larvae before starting rearing programs. In cooperation with the University of Kiel, we repeated the survey of their *T. jacobaeae* release experiment from 2017-2020 in 2023 to get a long-term perspective of their efficacy. Thereby, we found that *T. jacobaeae* could successfully reduce the number of generative *J. vulgaris*, as well as the number of its shoots. During the field season in 2024 we will set up semi-natural field experiments to determine the necessary application rate of larvae per plot for an effective regulation of *J. vulgaris*. Furthermore, experiments in the laboratory are ongoing to determine the potential of targeting alternative ragwort species like *Jacobaea aquatica* G. Gaertn., B. Mey. & Scherb and the invasive *Senecio inaequidens* D.C. for egg laying and larval feeding. Based on the current data, we believe that *T. jacobaeae* has some potential to regulate *J. vulgaris* and potentially even other species like *J. aquatica* and *S. inaequidens*, but releasing diseased, infected or parasitized larvae needs to be avoided in order to establish vital populations of this species.

P7-13

## **Nectar-inhabiting microbes affect olfactory responses of insect parasitoids**

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Floral nectar is frequently colonized by microbes among which bacteria and yeasts are the most abundant. These microbes have the ability to alter nectar characteristics with consequences not only for pollinators, but for the whole community of flower-visiting insects. Insect parasitoids are important biological control agents and are known to rely on sugar-rich resources – such as floral nectar - at the adult stage. In this study, we first isolated 14 nectar-inhabiting microbes from buckwheat (*Fagopyrum esculentum*) which is an important flowering plant used in Conservation Biological Control. Then we tested the microbe-mediated effects on the egg parasitoid *Trissolcus basalis* a biological control agent of the southern green stink bug *Nezara viridula*. In olfactometer studies coupled with gas-chromatography and mass spectrometry (GC-MS), we found that microbial fermentation of synthetic nectar triggered the emission of microbial volatile organic compounds which, in turn, affected parasitoid olfactory responses. Overall these findings highlight the need to consider the role of microbes when studying interactions between flower nectar and egg parasitoids and could have implications for developing conservation biological control programs



P7-15

## **Belowground herbivory differentially alters preference and performance of an aboveground parasitoid and generalist predator**

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While belowground herbivory often negatively affects parasitoid preference and performance on aboveground herbivores, little is known about how generalist predators respond and perform in these systems. Because generalists typically display reduced cue specificity compared to parasitoids and are not constrained by the host for development, we expected that parasitoids would prefer aphid hosts on plants without belowground damage, and would parasitize aphids on those plants at a higher rate. In contrast, we expected that the generalist lady beetle would not distinguish between aphids on either plant. We investigated these questions using a focal system of corn aphids (*Rhopalosiphum maidis*), the convergent lady beetle (*Hippodamia convergens*), and the parasitoid *Aphidius colemani* on maize (*Zea mays*). Specifically, we determined how the presence of Western corn rootworm (WCR) larvae altered aphid population growth, consumption by predators, parasitism rates, and natural enemy preference. We also analyzed how the presence of WCR and aphids altered aboveground maize volatile blends after 24h and 1 week of aphid herbivory. Feeding by WCR larvae belowground reduced aphid population size aboveground after 2 weeks of feeding. In contrast to our predictions, *A. colemani* produced equal number of mummies per female between the two treatments, while lady beetles consumed more aphids on control plants. However, *A. colemani* preferred plants with aphids alone over control plants after 24h of aphid feeding, but did not distinguish between other pairwise comparisons, while *H. convergens* did not distinguish between different herbivory treatments at 24h or 1 week in choice assays. Analysis of aboveground volatiles demonstrated that at both timepoints the combination of above- and belowground herbivory explained more of the variation in volatile blends than either above- or belowground herbivory alone. While most previous work has focused on the effect of belowground herbivory on parasitoids, our results demonstrate the importance of considering generalist predators as well and suggest that better understanding the effects of natural enemies in combination could yield a more accurate understanding of population dynamics in systems with multiple herbivory.

P7-16

## Plant-mediated effect of *Cotesia glomerata* polydnavirus and parasitism on parasitoid recruitment

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Plants are in a constant battle with insect herbivores. When plants perceive insect-derived cues like feeding damage, oviposition and oral secretions, they often enhance their defences to the specific herbivore. This induced defence can be direct by reducing herbivore performance, e.g. through the activation or production of toxic compounds like glucosinolates in cabbage. Alternatively, the plant can mount indirect defences by recruiting natural enemies of herbivores that reduce the impact of herbivory. One of those indirect defences is the production of herbivore-induced plant volatiles. These volatiles can be attractive to a wide array of other insects in the third trophic level, like parasitoid wasps. These are wasps that lay their eggs inside herbivores, which makes them effective biocontrol agents.

We have a lot of knowledge about how herbivores influence their food plant and the insect-plant community. However, little is known about what happens with these interactions once parasitoid wasps have parasitized these herbivores. Recent studies have identified that parasitoids can have a great, top-down plant-mediated effect on the community through both behavioural- as well as physiological changes in their host.

*Cotesia glomerata* is a parasitoid wasp that carries a polydnavirus (CgPDV). Along with injection of eggs, the parasitoid infects its caterpillar host *Pieris brassicae* with CgPDV, resulting in physiological changes in the caterpillar that benefit the offspring of parasitoids that develop inside the caterpillars. At the same time changes in the physiology of the parasitized caterpillar influence the defensive response of its food-plant and thereby interactions with other community members.

So far, it is known that plants associated with CgPDV-infected caterpillars repel the moths of *Plutella xylostella* and attract the hyperparasitoid wasp *Lysibia nana*. This field season, I am investigating whether this altered plant response results in differential attractiveness to both conspecific parasitoid wasps as well as parasitoids of different species. I will research this both in the lab as well as in the field. Additionally, I will investigate how the volatile blend is altered due to herbivory by CgPDV-infected *Pieris brassicae*.

With this research, I will demonstrate that parasitoid wasps and their symbionts can have a significant, plant-mediated ecological effect on the rest of the insect-plant community.

P7-17

## Dual effects of above- and belowground plant mutualists on insect herbivory

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Plant associations with multiple types of mutualists often implicate above- and belowground interactions, among which ant-plant interactions and arbuscular mycorrhizal fungi (AMF) are two prominent cases. Whereas each mutualism has been well studied on its own, studies testing for both interactions simultaneously and the potential for non-additive effects on plants remain scarce, and the plant traits mediating such dynamics are often neglected. To address this gap, we conducted a joint test of AMF and ant effects on insect leaf herbivory on potato (*Solanum tuberosum*) plants, and further assessed candidate plant traits mediating mutualist effects on herbivory. We conducted a factorial field experiment manipulating the presence of AMF (two levels: control and mycorrhization) and ants (two levels: exclusion and presence) and tested for AMF effects on phenolic compounds acting as direct defences, as well as on volatile organic compounds (VOCs) potentially mediating direct (e.g., herbivore repellents) or indirect (e.g., ant attractants) defence. To further understand AMF effects via indirect defence and potential non-additive dynamics, we measured ant abundance in the field and performed a dual-choice greenhouse experiment testing for effects of VOC blends (mimicking those emitted by control vs AMF-inoculated plants) on ant attraction. Whereas mycorrhization had no detectable influence on herbivory, ants significantly reduced leaf damage. Mycorrhization had no effect on leaf phenolics, but increased VOC emissions. Nonetheless, it did not lead to increased ant abundance, and there was no evidence for mutualist interactive effects on herbivory via ant-mediated defence. Consistently, the dual-choice assay showed no effect of AMF-induced VOC blends on ant attraction. Overall, this study provides a novel test of linkages between below- and aboveground plant mutualisms affecting herbivory and sheds mechanistic insight into interaction outcomes by measuring plant traits underlying such effects.

Keywords: Ants, arbuscular mycorrhizal fungi, phenolic compounds, plant defences, *Solanum tuberosum*, volatile organic compounds

P7-18

### Augmenting Bt pest control using a synthetic leaf microbiome

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*Bacillus thuringiensis* (Bt) is a leading biological pest control agent against Lepidoptera pests. While effective, its efficacy can be compromised by its limited persistence on leaves and hence the timing of application is crucial. Consequently, there is scope to improve Bt's efficacy, which could further increase its adoption and reduce farmers' reliance on harmful chemical pesticides. Building on studies suggesting that commensal bacteria in the larval gut impact Bt efficacy, we investigated whether plants inoculated with a specific bacterial community (patent pending) that colonise Brassicaceae leaves enhances the efficacy of Bt against larvae of the Large White butterfly, *Pieris brassicae*. Using a gnotobiotic plant system, we placed neonate larvae on *Arabidopsis thaliana* plants that were either inoculated with the bacterial community or kept bacteria-free. After seven days, larvae from both groups were exposed to a sublethal dose of commercial Bt. Four days after Bt exposure, larvae that fed on plants inoculated with the bacterial community showed 100% mortality, compared to a mean mortality of 23% in larvae that fed on mock-treated plants. Larvae that fed on plants treated with only the bacterial community, but not Bt, experienced no increase in mortality. We investigated the mechanism behind the enhanced Bt lethality and found that larvae feeding on plants inoculated with the bacterial community, and then exposed to Bt, exhibited significantly higher bacterial numbers in their haemolymph compared to larvae that fed on bacteria-free plants before Bt exposure. This suggests that the enhanced efficacy of Bt, when used in combination with the bacterial community, arises from a synergistic effect, where rapid haemolymph infection by both Bt and bacteria from the community leads to quicker larval death. Our study demonstrates that Bt efficacy can be substantially enhanced by specific leaf bacterial communities, offering a promising eco-friendly method to boost the efficacy of this biological control agent.

P7-19

**Interaction between the entomopathogenic fungus *Beauveria bassiana*, tomato plant and the Lepidopteran pest *Spodoptera littoralis*: Mechanisms and consequences in agricultural plant protection**

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Beneficial microorganisms are able to promote plant growth and defence barriers, offering a valuable alternative to synthetic agrochemicals. *Beauveria bassiana* is one of the most important entomopathogenic fungi that can colonize a wide variety of plant species as an endophyte, limiting the growth and survival of plant pests and pathogens. Here we contribute to this research topic by studying the effect of tomato plants colonization by *B. bassiana* on the survival, development, and immunity of *Spodoptera littoralis* (Lepidoptera, Noctuidae). Endophytic colonization of plants did not affect the survival of larvae feeding on them, which showed a weight increase associated with a higher pupal mortality and a lower adult fecundity. Interestingly, encapsulation and nodulation responses of larvae fed with *B. bassiana* colonized plants were reduced, while, in contrast, phagocytosis slightly increased. These larvae proved to be more sensitive to *B. bassiana* or *Bacillus thuringiensis* infection, given their reduced immune competence. "Omic" studies on tomato plants and *S. littoralis* larvae aim to unravel the molecular mechanisms underlying these changes. Our study provides key findings toward the understanding of mechanisms underlying this intricate plant-insect-microbe interaction interconnecting the below-ground and above-ground environments. It also sheds light on the evolution of the entomopathogenic lifestyle in soil fungi.

P7-20

### **Root colonization by fungal entomopathogen systemically primes belowground plant defense against cabbage root fly**

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Entomopathogenic fungi infect insects via spores but also live inside plant tissues as endophytes. Frequently, colonization by entomopathogens provides plants with increased resistance against insects, but the mechanisms are little understood. This study investigated direct, local, and systemic root-mediated interactions between isolates of the fungus *Metarhizium brunneum* and larvae of the cabbage root fly (CRF) *Delia radicum* attacking *Brassica napus* plants. All fungal isolates infected CRF when conidia were present in the soil, leading to 43–93% mortality. Locally, root-associated *M. brunneum* isolates reduced herbivore damage by 10–20% and in three out of five isolates caused significant insect mortality due to plant-mediated and/or direct effects. A split-root experiment with isolate Gd12 also demonstrated systemic plant resistance with significantly reduced root collar damage by CRF. LC-MS analyses showed that fungal root colonization did not induce changes in phytohormones, while herbivory increased jasmonic acid (JA) and glucosinolate concentrations. Proteinase inhibitor gene expression was also increased. Fungal colonization, however, primed herbivore-induced JA and the expression of the JA-responsive plant defensin 1.2 (PDF1.2) gene. We conclude that root-associated *M. brunneum* benefits plant health through multiple mechanisms, such as the direct infection of insects, as well as the local and systemic priming of the JA pathway

P7-21

## **Manipulation of the soil microbiome to enhance insect pest suppression in crop plants**

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Beneficial soil microbes can promote plant growth and induce plant resistance against insect pests. Combining multiple beneficial microbes into synthetic communities designed to simultaneously increase plant health and yield has a strong potential to support sustainable agriculture. My research aims to uncover the mechanisms behind these plant-microbe-insect interactions, seeking to optimise and boost plant health. Inoculation of barley roots with *Acidovorax radialis* and *Bacillus subtilis* shows aphid suppression in lab and field trials. Data from the field trial shows that plant rhizosphere microbial communities varied due to inoculation treatments, barley variety, and experimental year. *A. radialis* bacteria communicate using quorum sensing, and our experimental results using quorum-sensing mutants indicate that these molecules can modulate plant-bacteria interactions in barley. We use our system to explore the relative effects of direct bacteria-plant interactions compared to indirect interactions via the wider microbiome, with the aim to determine what key factors are needed to develop synthetic crop microbial communities. Understanding how beneficial microbes interact with the plant and other microbes will enable the development of resilient microbial communities that offer protection across multiple crops and environments.

P7-22

## **Impact of insect-suppressing microbial inoculants on barley grain quality.**

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Pest control in crop plants is of significant scientific and economic interest, in line with reducing the use of environmentally harmful agrochemicals. Microbial inoculation of crops to promote plant growth and enhance plant defences against insect pests is a potential alternative to wide-spread use of pesticides or fertilizers. While studies have shown promising results for crop yields and reduction of pests, many ignore potential effects on the quality of the crop. In cereal crops, such as barley that are used for distilling and brewing, a change in grain quality and composition may impact final products. I examined this using barley plants (*Hordeum vulgare*) inoculated with known pest suppressing microbes (*Acidovorax radialis* and *Bacillus subtilis*) and the presence/absence of aphids (*Sitobion avenae*). I will present data demonstrating observed effects on barley grain quality, with specific reference to quality for brewing. Understanding yield quality effects of microbial-based products is a vital step to delivering sustainable alternatives to farmers.

P7-23

## Does the phytopathogen *Fusarium oxysporum* modulate the olfactory behavior of the banana weevil?

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Bananas are susceptible to various pests and diseases, posing considerable challenges to their production. The main pest of banana plants is the banana weevil *Cosmopolites sordidus*, found in almost all banana-producing areas. This weevil opens galleries in the banana plant's rhizome, facilitating the entry of pathogens. *Fusarium oxysporum* f. sp. cubense is the causal agent of banana wilt, also known as "Panama disease". This fungus can survive in the soil even in the absence of the host and can be transmitted from an infected plant to a healthy one in various ways. Recent work with fungi of the genus *Fusarium* identified that this pathogen manipulates the pathosystem to promote its dissemination. Therefore, the objective was to verify and elucidate the presence of a manipulation system in the interaction between banana, *C. sordidus*, and *F. oxysporum*, through studies of volatile organic compounds (VOCs) present in the plant-pathogen interaction using mass spectrometry (GC/MS) and their effects on insect behavior. Cavendish banana plants were inoculated with *Fusarium oxysporum* f. sp. cubense Tropical Race 4 (Foc TR4), with simulated plants inoculated with a solution without conidia, and maintained under controlled conditions. Choice preference test was carried out using a four-chamber olfactometer and offering infected and uninfected roots or soil to the beetles. To collect and quantify VOCs, Tenax traps tubes were inserted into the soil of each treatment. Chemical identification was carried out using gas chromatography coupled with a mass spectrometer. The results of preferences and VOC analysis are still ongoing. We expect that the pathogen manipulates the insect, and additionally, there is a manipulation mechanism preserved in fungi of the genus *Fusarium*.



P7-24

## Trichoderma-induced metabolic changes in maize and their effects on higher trophic organisms

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The intricate interactions between crops and root-colonizing endophytic fungi such as *Trichoderma* spp. are intensively studied as they can enhance plant resistance to pests and boost crop yields. Fungi in this genus produce a wealth of specialized volatile and non-volatile metabolites but their ecological functions are not well understood. Previous studies have shown that colonization of maize roots by *Trichoderma virens* wild-type (WT) and its knockout mutant *vir4* (deficient in the genes cluster responsible for the biosynthesis of sesquiterpenes) can modulate the transcriptome and metabolic profile of maize (Schweiger et al. 2021). Following up on this, we tested whether these changes can alter the quality of maize leaves as a food source for caterpillars of *Helicoverpa armigera* (Lepidoptera, Noctuidae). Bioassays with maize seedlings colonized by either WT or *vir4* mutant strains revealed negative impacts on the development of *H. armigera*. However, maize colonized by the WT strain resulted in more significantly attenuated caterpillars development than maize colonized by the *vir4* mutant. Untargeted LC-MS/MS metabolite profiling of maize treated with either fungal genotype or with herbivory showed that both genotypes had weak effects on the leaf metabolome when compared to herbivory. In herbivore-damaged maize, fungal colonization by WT and *vir4* resulted in different metabolic phenotypes. Taken together, these results suggest a role for the *vir4* gene cluster in mediating enhanced resistance in *T. virens* colonized maize.

Schweiger R., Padilla-Arizmendi, F., Nogueira-López, G., Rostás, M., Lawry, R., Brown, C., Hampton, J., Steyaert, J.M., Müller, C., Mendoza-Mendoza, A. (2021) Insights into metabolic changes caused by the *Trichoderma virens*–maize root interaction. *Molecular Plant-Microbe Interactions*, 34: 524-537. <https://doi.org/10.1094/MPMI-04-20-0081-R>

P7-25

## Semiochemical-based alternative concepts for the management of wireworms

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Wireworms (larvae of click beetles) are polyphagous soil pests that significantly impact cereal and potato crops in Europe and North America. Studies have reported that wireworms cause 10-37% seedling mortality in cereals in greenhouse conditions [1], and they reduce harvest quality by 10-60% in other crops [2]. Stringent pesticide regulations and a shift towards sustainable practices, including minimal tillage, have inadvertently increased wireworm infestations. Thus, sustainable pest management strategies, including using semiochemicals, are necessary.

Semiochemicals (naturally occurring development- and behaviour-modifying chemicals) provide an environmentally benign alternative for pest management due to their non-toxic mode of action. Like aboveground pests, soil herbivores are also attracted to or repelled by semiochemicals that occur in the gas phase and diffuse in soil pores. While carbon dioxide (CO<sub>2</sub>) generally attracts wireworms in the rhizosphere, its effectiveness as a specific cue for locating host plants within diverse plant communities remains uncertain. This project investigates whether root-emitted volatile organic compounds (VOCs) provide precise cues for wireworms to locate their hosts and assesses their potential as targets for control practices.

Wireworms showed a behavioural preference for certain wheat varieties in soil olfactometer assays, whereas others did not attract them. This indicates that soil herbivores use root VOCs as cues to locate their hosts and can differentiate between varieties of the same species. Future work will focus on evaluating the root VOCs of these varieties and identifying active compounds that elicit a behavioural response in wireworms.

The identified compounds, applied as slow-release formulas, may be used as a stimulo-deterrent diversion in a push-pull system and for monitoring wireworm populations in agricultural fields. Furthermore, it will create a model for other sustainable crop protection practices, including breeding programmes with desirable pest resistance traits.

[1] Arcillas, L. S. (2022). <https://thesis.unipd.it/handle/20.500.12608/60252>

[2] Epi-Agri (2022). <https://bit.ly/3QSfMgS>

P7-26

## Environmental DNA deposition reflect insect taxon, abundance and insect-plant interaction times

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There is a variety of tools used to monitor and survey biodiversity and abundance of insects and other organisms. However, many of these monitoring tools are not able to reveal ecological networks or food webs. eDNA metabarcoding holds a great potential as a simple and efficient tool to detect such networks as for instance plant-insect interactions. However, the influence of both abiotic and biotic factors on eDNA deposition in plant-insect interactions is not well understood. It is still unclear, if deposited eDNA quantities reflect the interaction time or number of interactions between insects and plants?

We experimentally analyzed the quantitative deposition of eDNA from three insect species (the southern green stink bug *Nezara viridula*, the mustard beetle *Phaedon cochleariae* and the fall armyworm *Spodoptera frugiperda*) on leaves of two host plants of varying attractiveness: savoy cabbage (*Brassica oleracea* var. *sabauda*, preferred host) and tomato (*Solanum lycopersicum*, less preferred host). We tested for the effects the host plant, exposure time and abundances of the different interacting insect species had on eDNA deposition. To eliminate as many environmental variables as possible, we conducted the experiments with plant material in petri dishes.

Our data clearly show a differentiated pattern of eDNA deposition. Insect abundance and interaction with plant material influence the amount of deposited eDNA. Moreover, there are significant differences of eDNA amounts between the host plants, so there are more variables in the deposition of eDNA as the insect alone. We found that generally more eDNA was deposited on cabbage than on tomato. However, there were strong differences between the insect species in their respective eDNA depositions as well.

Our results suggest that eDNA could be used as a quantitative and not only qualitatively monitoring tool once we have better understood specific patterns of eDNA deposition. Identifying specific differences in eDNA abundance and deposition time from different insect-plant interactions is a big step forward to a holistic monitoring approach, which could enable the possibility to study multi-trophic, as well as single insect-plant interactions, in nearly real-time.

P7-27

## **Toward the use of environmental DNA (eDNA) for rapeseed (*Brassica napus* L.) arthropod pest monitoring**

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The usage of environmental DNA (eDNA) is greatly revolutionizing the field of biomonitoring. By using DNA shed by organisms and released into the surrounding environment, it is possible to consider if a species is present or not. Such biomonitoring usage has been the main focus of eDNA studies, however, in recent years eDNA has also been used for trying to estimate species abundances and plant-animal interactions: specifically, animals release DNA when interacting with plants and such animal DNA can be extracted from plant material. Furthermore, it may be possible to estimate the usage and the intensity of usage of a specific plant by an animal by examining the abundance of target reads of such animal species.

Species detection and their abundances' estimates can also be useful in an agricultural setting, specifically, plant pests can be detected by extracting eDNA from plant themselves or even by analyzing eDNA collected passively (e.g., through air sampling) in agricultural areas. This could be useful for facilitating pest detection and management before the infestation becomes evident (i.e., visible), given the power of eDNA of detecting species at early non-evident (e.g., larvae inside plants) stages or when their abundance is still low.

For evaluating the potential usage of eDNA for arthropod pest monitoring, we designed a weekly field sampling trial in three different rapeseed (*Brassica napus*) fields in Luxembourg, rapeseed is an important crop species in Central Europe, and the selected fields have been monitored for arthropod pest species by local researchers for the past twenty years. We will use different sampling strategies (both traditional and eDNA ones), and we will compare the abundance data obtained from eDNA to those collected previously using yellow pan traps twice the week from February to mid of May. This comparison will help determine if eDNA yields similar or differing conclusions compared to traditional sampling methods.

This study will be important for developing tools for studying plant-arthropod interactions and for quantifying pests in an agricultural setting. In addition, our data may potentially help developing new technologies or standardizing existing ones, such as portable devices for detecting target species or air sampling for eDNA research.

P7-28

## A test system for herbivorous species: Pre-tests and challenges

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In the EU, the risk assessment for non-target arthropods (NTAs) follows the framework developed by ecotoxicologists in the ESCORT workshops. In 2015 EFSA published a Scientific Opinion (SO) to address the request of the EU commission to review the existing framework. Different ways of exposure to pesticides are one of the aspects that were addressed in the SO asking for an additional test species. In particular, the oral exposure through ingestion of contaminated food was identified as a challenge since the current available endpoints from a tier 1 test design cannot be related to oral toxicity and cannot be used in a risk assessment for this exposure mode. Therefore, the working group of the PPR panel recommended to develop assessment methodologies for focal species such as herbivorous NTAs. Hence, there is the need to develop a reproducible methodology to enable us to evaluate the oral toxicity of PPP to lepidopteran larvae, for example.

Additional to other initiatives followed by the CLE NTA working group, we started working with one common lepidopteran species to gain first experiences which will guide the further development of methodologies. The project's purpose is to develop a test method to be used under Good Laboratory Practice (GLP) in the laboratory. Criteria for the selection of test species were defined and *Plutella xylostella* (Plutellidae, Lepidoptera) was identified as a suitable test organism. Different exposure matrices and diets were tested during first trials. Key factors related to validity criteria like control mortality, Larval feeding behavior, larval development time, adult hatching rate and egg production in the control were evaluated.

The advantages and disadvantages of *P. xylostella* as an organism for which laboratory-based breeding techniques are well established, also for efficacy trials, will be highlighted.

We will provide first recommendations for the development of a testing protocol and present results to illustrate the technical feasibility and expected challenges in developing assessment methodologies for oral exposure using an herbivorous arthropod like *P. xylostella*.

P7-29

## Lights, camera, action! Investigating aphid resistance in peppers through automated video tracking.

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Aphids are among the most threatening pests of pepper (*Capsicum* spp.) cultivation in the Netherlands with the most important species being the green peach aphid (*Myzus persicae*). Aphids are important pests as they can spread plant viruses and are detrimental to crop production. Recently, intensive insecticide use has led to the emergence of insecticide-resistant *M. persicae* genotypes. Therefore, finding alternative ways for aphid control is of great importance. Insufficient opportunities have been exploited to breed for plant resistance and a fundamental understanding of aphid-pepper interactions in cultivated *Capsicum* spp. is lacking. Especially species within the Pubescens and Purple corolla clade have been understudied. We aim to use the genetic diversity in these relatives of *C. annuum* to identify resistance mechanisms against *M. persicae*.

We quantified aphid feeding behaviour in a panel of 14 *Capsicum* spp. accessions with the EntoLab™ automated video-tracking system (Noldus Information Technology, Jongsma et al. 2019, Kloth et al., 2015). This high-throughput automated video-tracking platform allows the screening of a maximum of 144 aphids in parallel on detached *Capsicum* leaves. Changes in pixel intensities across video frames were analysed by software algorithms and used to detect the aphids and determine their location over time (Dell et al., 2014, Noldus et al., 2002). The data were used to identify aphid movement patterns, which are indicators of aphid probing behaviour (Kloth et al., 2015).

We assessed natural variation in resistance among accessions of *C. annuum*, *C. baccatum*, *C. cardenasii*, *C. eximium*, and *C. pubescens*. Screening of 14 *Capsicum* spp. accessions resulted in the identification of one *C. pubescens* accession where aphids showed more short (unsuccessful) probes and more movement compared to aphids on the susceptible *C. annuum* accession. By using this method, we were able to quickly identify interesting accessions to study in more detail in the future.

P7-30

## Catch me if you can! Biobased glues for crop protection against insect pests

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Arthropod pests are responsible for billions of dollar's worth of agricultural yield losses every year. The chemical pesticides used to control arthropods are associated with environmental pollution and health risks for many organisms. Additionally, these chemicals are also losing effectiveness due to increasing pest resistance. More sustainable methods of pest control are clearly needed. Inspired by carnivorous plants that trap insects with sticky fluids, we created sprayable solutions containing sticky droplets made from oxidized plant oils to trap one of the most severe invasive pests in horticulture and ornamentals: western flower thrips.

We performed thrips adhesion bioassays and full-plant experiments with *Chrysanthemum* (an economically significant ornamental crop) to investigate if adhesive droplets made from different plant oils could catch thrips and reduce thrips damage. In addition, we used nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GC-MS) to investigate the metabolomic response of *Chrysanthemum* to application of our adhesive droplets.

We will show that spraying plants with solutions containing adhesive droplets can trap thrips and reduce thrips damage and reproduction. Both the application of the solution with adhesive droplets and thrips presence affected the plant metabolome, and led to increased concentrations of chemicals in sprayed and unsprayed leaves that can be herbivore repellent or toxic. Our study, where we use sticky plant-based pesticides to protect crops from tiny arthropod pests, provides an example of a "nature-based solution" that can be used to improve sustainability in agriculture.

P8-01

## Evolutionary and experimental insights into the functional diversity of complex trichomes in Loasaceae

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Trichomes are morphologically diverse epidermal structures that are expected to vary within plant lineages in response to differing selection pressures across ecological and environmental gradients. Nonetheless, despite the incredible variation present in this trait, we lack experimental tests of function for many trichome types and know even less about the factors shaping trichome diversity on a macroevolutionary scale. Our work integrates experimental and comparative methods to examine the function and evolution of trichomes in the Loasaceae, a family known for its complex hairs. We conducted experiments testing how complex barbed and needlelike trichomes in *Eucnide* impact a generalist herbivore and investigated patterns and potential drivers of fine scale trichome investment across the genus *Mentzelia*. We found that barbed trichomes on *Eucnide urens* leaves severely immobilize *Spodoptera exigua* caterpillars and that removing the trichomes significantly increases caterpillar feeding and growth. Looking across 53 species of *Mentzelia*, we found that patterns of trichome investment contradict classic hypotheses for aridity and latitude and uncovered support for the hypotheses that *Mentzelia* species with larger flowers shift investment away from sticky barbed trichomes, potentially reducing defense-pollinator conflict. Importantly, our analyses revealed different evolutionary patterns for different trichome traits, highlighting the value of incorporating multiple axes of diversity when addressing questions about the adaptive roles of trichomes across lineages. Together, these results shed light on longstanding questions about trichome functional diversity and invite further work examining the ecological and evolutionary implications of trichome variation across lineages.



P8-02

## Maintenance of induced resistance upon competitor perception in a densely growing perennial plant

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Considered to be “the dilemma of plants” one classically hypothesized tradeoff is “to grow or to defend”, or more specifically between investing into competitive ability or defense against herbivores and pathogens. The artificial uncoupling of the growth-defense trade-off in recent studies suggests that selection may act on these traits to naturally uncouple growth and defense, possibly allowing for equal phenotypic expression of either function (i.e. not only growth or only defense). Previous work examining the relationship between induced resistance and competitor perception has been conducted with early pioneering annual species, which are hypothesized to prioritize competitive ability over defense due to the strong temporal limit on their lifetime growth. It remains elusive, however, if non-annual plants invest differently along the growth-defense trade-off axes due to their contrasting life history. One system for which a natural uncoupling of the growth-defense tradeoff can be predicted is *Solidago altissima* for which insect herbivores are a large selective agent. Insect herbivory in *S. altissima* has been shown to directly affect competitive outcomes to the extent that the removal of herbivores changes plant community composition and succession dynamics. Accordingly, insect herbivory has been shown to act as selective agents on constitutive and induced resistance, as well as competitive ability. Here we test the hypothesis that the relationship between insect herbivores and competitive ability in *S. altissima* is such that the ecology and life history of the plant favors the maintenance of induced resistance in the presence of potentially competitive neighbors. We predicted that despite phenotypic changes characteristic of neighbor perception, such as elongating growth, shown to result in a downregulation of induced chemical defenses, *S. altissima* would maintain its ability to induce chemical resistance to specialist insect herbivores. With a combination of greenhouse experiments, insect bioassays, and tissue secondary metabolite analysis, we demonstrate that contrary to the predictions of the growth-defense hypothesis and previous findings, *S. altissima* not only maintains its ability to induce resistance, but actually the effect of induction is stronger in the presence of a neighbor. Moreover, we demonstrate that at least for one defensive compound class, induction is only evident in the neighbor-perceiving plants.

P8-03

### Joint effects of inter-specific and intra-specific diversity of tomato volatile profiles on antixenosis against *Tuta absoluta*

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Since volatile organic compounds (VOCs) shape plant defenses against pests, ecologists strive to understand their sources of variation. The worldwide distribution of tomato offers an ideal system to explore the ecological diversity of VOCs produced by plants supporting a large range of local adaptations. In this study, we analyzed the diversity of VOC profiles released by 29 accessions of tomato belonging to five species with different natural histories and including domesticated plants and wild relatives. In addition, olfactometer assays were performed on 10 accessions characterized by strong differences in VOC emissions to test the consequences on host-plant location by a major pest specialized on solanaceous plants, *Tuta absoluta*. Based on 77 VOCs detected, our results suggest that the large diversity of volatile profiles reflect different biogeographic areas. A phylogenetic tree was constructed to test phylogenetic drivers in VOC emissions. The richness of volatile profiles and sesquiterpenoid emissions were relatively high in ancestral branches and decreased exponentially with evolution according to early-burst patterns. In olfactometer assays, we identified four repellent accessions belonging to *S. pennellii*, *S. habrochaites* and *S. cheesmaniae*. This study points out the importance of inter- and intra-specific diversity of VOCs in antixenosis. Further, our results suggest that methyl salicylate,  $\beta$ -myrcene and sesquiterpenoids such as (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene or  $\alpha$ -curcumene could serve as repellents against *T. absoluta*. In a greenhouse, we confirmed the biological relevance of agroecological research interested in screening repellent plants. Bridging chemical ecology with phylogenetic approaches helps to better understand the evolution of mechanisms supporting anti-herbivore plant defenses.

P8-04

## Aboveground JA- and SA-pathways induce stress-specific root microbiome responses in *Brassica oleracea*

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Induction of plant defense responses aboveground has been linked to changes in belowground root microbial communities, but it remains unclear if these changes are stress-specific and beneficial for plants. In this study, we conducted a plant-soil feedback (PSF) experiment to explore whether root microbiome shifts are specific to Jasmonic Acid (JA) and Salicylic Acid (SA) phytohormonal defense signaling pathways.

In the conditioning phase, we induced the JA and SA pathways in *Brassica oleracea* shoots by infesting plants with insect herbivores or by simulating herbivory through the application of phytohormones. Simulated herbivory involved treating plants with Methyl Jasmonate (MeJA) or SA. For infestation, plants were subjected to *Mamestra brassicae* caterpillars (chewers) or *Myzus persicae* aphids (phloem-feeders). We analyzed the rhizosphere bacterial communities via amplicon sequencing, assessed the phytohormone and glucosinolate profiles of the shoots, and collected conditioned soils as inocula.

In the feedback phase, we grew a second generation of *B. oleracea* plants in the six types of soil inocula (JA-conditioned, SA-conditioned, and control-conditioned from both insect and simulated herbivory) and exposed plants to *M. brassicae*, *M. persicae*, or kept them uninfested to assess resistance. Results confirmed that JA and SA pathway induction was similar between simulated and real herbivory, as phytohormone and glucosinolate profiles were comparable.

Root microbial communities changed specifically according to the defense pathway induced. Proteobacteria decreased in response to MeJA treatment and chewing herbivory (JA-induced), but not SA and aphid herbivory (SA-induced), while Planctomycetota were enriched in both pathways, suggesting a general stress response. Despite changes in the root microbiome, there was no effect of soil inocula on plant growth or insect resistance during the feedback phase. This study provides evidence that plant defense induction alters root microbiome communities in a stress-specific manner.

P8-05

## Herbivory promotes insect-specific soil legacies that impact future plant resistance in *Baccharis salicifolia*

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Herbivory can alter the soil microbiome and create legacy effects that impact future plant resistance via plant-soil feedbacks. However, whether these effects vary between leaf chewing vs. sap feeding herbivores, and the underlying mechanisms via plant traits, remain largely unexplored. Here, we investigated whether herbivory by generalist leaf chewing caterpillars (*Spodoptera exigua*) and sap feeding aphids (*Aphis gossypii*) creates herbivore-specific soil legacies that affect plant resistance, using mulefat (*Baccharis salicifolia*) as a model species. We also explored the plant traits underlying such legacy effects, focusing on leaf chemical defences (phenolic compounds) and nutritional quality (carbon and nitrogen content). We conducted a greenhouse experiment in two steps. Firstly, we conditioned soil with herbivory on *B. salicifolia* plants under three treatments: (1) soil conditioned by caterpillar herbivory, (2) soil conditioned by aphid herbivory, and (3) control soil not conditioned by insect herbivory. Secondly, we grew a different set of *B. salicifolia* plants in sterile soil mixed with inoculum consisting of 10% of conditioned soil from each treatment. Subsequently, we conducted resistance bioassays on these plants to assess the performance of both herbivores and collected leaves (pre and post-bioassay) to measure plant traits. We found that herbivory by *S. exigua*, not by *A. gossypii*, created legacy effects. Specifically, *B. salicifolia* plants growing in soil conditioned by caterpillar herbivory exhibited a 12% higher C:N ratio, indicative of lower tissue nutritional quality, and a trend towards increased inducibility of phenolics compared to plants growing in control soil. In turn, plants growing in soil conditioned by caterpillar herbivory showed reduced performance of *A. gossypii*, with approximately 17% lower aphid colony size than plants growing in control soil. Accordingly, aphid colony size was negatively associated with C:N ratio. Legacy effects of *S. exigua* herbivory on plant traits did not lead to significant differences in resistance to *S. exigua* on feedback plants. Because these legacies occurred within the context of soil inoculation, they were likely driven, at least in part, by changes in soil microbiome communities. Our results suggest that herbivory-induced soil legacies on plant resistance are herbivore-specific, both in terms of soil conditioning and the resulting induced resistance.

P8-06

## Artificial intelligence as a possibility for pest prediction in forests - Case study nun moth (*Lymantria monacha*)

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The increasing relevance of potential forest pests driven by climate change is also evident in the nun moth (*Lymantria monacha*), one of the most important pests on pine trees (Hentschel et al. 2018). Despite its polyphagous nutrition, coniferous stands are preferred, exposing for example the federal state of Brandenburg in Germany to increased vulnerability due to its large areas of pure pine stands (*Pinus sylvestris*) (Majunke et al. 2004). The larvae hatch in April and feed lavishly on needles until July causing potential severe damage on pine stands. Currently *L. monacha* is in a phase of progradation, which culminates in mass reproduction occurring every 10 years (Hentschel et al. 2017). The KINoPro project aims to use artificial intelligence (AI) to predict the occurrence of potential insect pests using the example of *L. monacha*. The objective of this case study is to improve current expensive monitoring procedures. AI is supposed to reduce the effort of time- and cost-intensive standard monitoring (Maaß et al. 2022), which consists of more than 700 traps in Brandenburg and more than 450 traps in Saxony. The AI uses trapping and weather data from the standardized monitoring of the federal states as training data generating an eight-week-forecast prior the first moth flight. The accuracy of this prediction is being optimized by additional monitoring data beyond the established monitoring area. First results show a considerable improvement of AI predictions from the first to the second year of the KINoPro project.

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P8-07

## **The effects of ungulate browsing on insect communities and trophic interactions between phytophagous and parasitoid insects in forest ecosystems**

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High ungulate densities can have significant effects on forest vegetation, leading to shifts in habitat structure and composition [1]. Their browsing behaviour can particularly impact natural regeneration, biomass development, and plant diversity, reducing the share of certain plant species [2]. Moreover, large ungulate populations can affect higher trophic levels in forest ecosystems [3]. In insect food webs, e.g., host-parasitoid interactions can be indirectly influenced by ungulate browsing. These cascading effects can modify the natural regulation of phytophagous insects by potentially beneficial parasitoid and predatory insects, thus, altering ecosystem stability [4].

The WiWaldI project analyses the consequences of ungulate browsing for insect food webs in forest ecosystems. The study focuses on trophic interactions between phytophagous, parasitoid and predatory insects. Insect habitats and communities will be identified at selected locations in Germany with different climatic, geological and forestry characteristics. The browsing effects will be determined by comparing unfenced and fenced plots. To analyse the insect communities, malaise traps and window flight traps were installed. Beating sheets were used on tree saplings of palatable species to identify insect communities specific to shrubs and tree species, as well as host plants.

The findings should contribute to the understanding of the interactions between ungulate populations and ecosystem functions in forests. Additionally, the study can provide habitat indicators for potentially beneficial insects, which could lead to practical recommendations for the biological control of phytophagous insects and the development of resilient and adaptable forests.

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[2] Vor, T., Ammer, C. (2021): Das BioWild-Projekt. Vegetationsentwicklung unter Wildeinfluss. *Ökojagd* 4, 19-24.

[3] Schulze, E. D., Bouriaud, O., Wäldchen, J. et al. (2014): Ungulate browsing causes species loss in deciduous forests independent of community dynamics and silvicultural management in Central and Southeastern Europe. *Annals of Forest Research* 57 (2), 267-288.

[4] Allombert, S., Stockton, S., Martin, J.-L. (2005): A natural experiment on the impact of overabundant deer on forest invertebrates. *Conservation Biology* 19 (6), 1917-1929.

P8-08

## **Pine responses to sawfly pheromones: Effects on pine direct and indirect defences against pine sawflies**

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Insect pheromones have widely been studied for their role in insect communication, but our knowledge on their impact on plant responses and subsequent effects on herbivorous insects is surprisingly limited. Exposure of pine trees (*Pinus sylvestris*) to sawfly (*Diprion pini*) sex pheromones is known to enhance pine defence against sawfly eggs.

Here, we investigated (a) if sawfly females have evolved behavioural counter-adaptations, which protect their eggs from increased mortality on pheromone-primed trees. Additionally, we analysed whether the priming effect of sawfly sex pheromones on pine direct defence against eggs extends to (b) direct defence against sawfly larvae and/or (c) indirect defence against sawfly eggs by improved attraction of egg parasitoids to egg-laden pine. Ad (a): Sawfly females did not differentiate between the odour of pheromone-exposed and unexposed trees from a distance. However, a no-choice oviposition bioassay revealed that more unexposed than pheromone-exposed trees received eggs. Ad (b): Performance of larvae and their pupation success did not differ on pheromone-exposed and unexposed trees. Ad (c): Our study of the effects of pheromone exposure on indirect pine defence against the eggs revealed that *Closterocerus ruforum*, a key egg parasitoid of pine sawfly eggs, did not differentiate between odour from pheromone-exposed and unexposed trees. Furthermore, we chemically analysed the emission rates of key terpenoids known to be released from egg-induced pine and crucial for attraction of the egg parasitoid. The pheromone exposure did not significantly affect the emission of the studied key terpenoids, regardless of whether trees were laden with eggs or not. However, as was shown in previous studies, sawfly egg deposition induced the emission of (E)- $\beta$ -farnesene, a highly relevant sesquiterpene for attraction of the parasitoids to egg-laden pine.

Taken together, no evidence was found that exposure of pine to *D. pini* sex pheromones affects pine direct defence against the larvae. Neither did exposure of pine to the pheromones affect pine indirect defence against the eggs. These findings suggest that the effects of pheromone exposure on pine responses are limited to pine direct defence against the eggs. However, *D. pini* females might cope with the pheromone-primed direct defence against the eggs by more readily ovipositing on trees that had not been exposed to their pheromones.

P8-09

## Propolis: An analytical exploration of bee-generated bioactives

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Propolis resin, a complex mixture with antimycotic and antibiotic properties, is found in bee hives and has long been used as a natural remedy. Crafted diligently by bees from plant resins, wax, and their own secretions, propolis serves multiple functions within the hive: It acts as a sealant, reinforcing structural integrity and maintaining sterility. There has been an increasing number of studies focusing on propolis produced by different bee species as alternative complementary medicines. This is due to the growing recognition of its potential in treating various acute and chronic diseases [1]. Thanks to dedicated efforts of the research community, we know that flavonoids, abundant in propolis, contribute to its bioactivity. However, their structural diversity poses analytical challenges. Isomeric flavonoids, sharing the same mass-to-charge ratio ( $m/z$ ), often co-elute during liquid chromatography-mass spectrometry (LC-MS) analyses, hindering precise identification and quantification.

Ion mobility separation coupled to LC-MS (LC-TIMS-MS) introduces an additional analytical dimension for the identification of such co-eluting isomers. This technique improves the effective separation of co-eluting isomers. Our study demonstrates the successful disentanglement of isomeric flavonoids within complex propolis samples, including morine and quercetin. Additionally, we explore the value of collisional cross section (CCS) values, determined through trapped ion mobility spectrometry (TIMS), for unambiguous annotation. CCS values provide insights into the three-dimensional conformation and shape of ions or molecules, helping researchers to distinguish different molecular species. CCS values are reproducible between systems and provide additional information to LC-MS, making them valuable for unambiguous annotation.

In summary, we will highlight how LC-TIMS-MS can help to explore the intricate relationship between bees, plants, and propolis as basis for bioactive compound discovery.

[1] <https://www.mdpi.com/1420-3049/27/18/6120>



P8-10

## **Multiple signals increase the response of specific receivers in a carnivorous plant-prey interaction.**

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Many organisms use multiple signals (e.g. color, movement, sound, many types of chemical signals) to convey seemingly the same information. Many of these signals are potentially costly, yet the repeated evolution of multiple signaling across systems suggests an adaptive value. Using prey attraction by a carnivorous pitcher plant, we tested two hypotheses of how multiple signaling may be adaptive: 1) additional signals increase the reliability with which key receiver(s) will respond, and 2) different signals reach different receivers, increasing the diversity of the receiver response. We used artificial pitcher plant models with three different signals to examine the reliability and diversity hypotheses in a manipulative, full factorial field experiment. We found support for the reliability hypothesis, with several clades of prey insects showing a greater response to multiple signals.

P8-11

## **Plant growth promoting bacteria (PGPB) in strengthening plant tolerance towards biotic stress via alteration of antioxidant defense system**

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Insect herbivores (aphids) and plant-parasitic nematodes are global, economically devastating pests that cause severe threats to agricultural production worldwide. Although both the pests are spatially separated, they might indirectly interact with each other by modulating the plant nutrition and chemical defense. Management of these pests is a major concern as agrochemicals used as insecticides or nematicides harm the environment and plant health. The use of plant growth-promoting bacteria is an eco-friendly way to strengthen plant growth and resistance and support sustainable agricultural production. The present study was designed to investigate the effect of *Pseudomonas* sp. as a plant growth-promoting bacteria (PGPB) on potato (*Solanum tuberosum*) growth and defense responses under non-stress and stressed environment. We investigated how potato interactions with nematodes and aphids alone and in the presence of PGPB alter the reactive oxygen species (ROS) formation particularly hydrogen peroxide in the below and above-ground tissues. Due to the sessile nature different mechanisms are adapted by the plants to counteract this oxidative stress. One such mechanism is the upregulation of the antioxidant defence system which comprises of ROS scavenging enzymes and antioxidant molecules. In the study we also observed the modulation in the activities of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) in response to single (aphids or nematodes) and dual (aphids + nematodes) biotic stress with and without PGPB.

P8-12

## Unravelling rhizobacteria responses to biotic stress in potato cultivars

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Potato (*Solanum tuberosum*) is a member of the Solanaceae family revered as a global staple and the third most important noncereal crop in terms of human nutrition. In this project, we explore the untapped potential of Northern Sweden as a significant potato producer. Unlike their counterparts in more southerly latitudes, potato cultivation in these regions requires significantly fewer pesticides and additives. This advantage is attributed to the absence or delayed arrival of diseases during the growth season, leading to a diminished reliance on pest control measures. The use of beneficial microorganisms could be an environmentally sound option to increase crop yields and reduce disease incidence. Through our investigations, we aim to unravel the intricacies of direct and indirect stress responses, both above and below ground, thereby enhancing our comprehension of the potato plant's resilience and adaptability to pests and diseases. Our results reveal potential mechanism triggering the growth promotion and biocontrol effects of rhizobacteria under biotic stress and suggest use of rhizobacteria may deployed for the improvement of potato crop yield. Through innovative strategies, we aim to provide valuable insights, that support the development of a resilient agricultural landscape, extending beyond the borders of Northern Sweden.