ABSTRACTS:

Insect Microbe Interactions: Oral Presentations

In programme order

Session 4

Nutritional endosymbionts' Shikimate pathways are susceptible to glyphosate inhibition

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Glyphosate is widely used as a herbicide, but recent studies begin to reveal its detrimental side effects on animals by targeting the shikimate pathway of associated gut microorganisms. However, its impact on nutritional endosymbionts in insects remains poorly understood. Here, we sequenced the tiny, shikimate pathway encoding symbiont genome of the sawtoothed grain beetle *Oryzaephilus surinamensis*. Decreased titers of the aromatic amino acid tyrosine in symbiont-depleted beetles underscore the symbionts' ability to synthesize prephenate as the precursor for host tyrosine synthesis and its importance for cuticle sclerotization and melanization. Glyphosate exposure inhibited symbiont establishment during host development and abolished the mutualistic benefit on cuticle synthesis in adults, which could be partially rescued by dietary tyrosine supplementation. Furthermore, phylogenetic analyses indicate that the shikimate pathways of many nutritional endosymbionts likewise contain a glyphosate sensitive 5-enolpyruvylshikimate-3-phosphate synthase. These findings highlight the importance of symbiont-mediated tyrosine supplementation for cuticle biosynthesis in insects, but also paint an alarming scenario regarding the use of glyphosate in light of recent declines in insect populations.

Keywords: Bacteroidetes; cuticle; insect decline; Oryzaephilus surinamensis; symbiosis

Tritrophic interactions between plant pathogens and their vector insects mediated by primary and secondary plant metabolites

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Phloem feeding insects from the genus *Cacopsylla* (Hemiptera: Psyllidae) are often vectors of plant pathogenic bacteria (phytoplasmas) causing severe diseases in fruit crops. We compared two closely related pathosystems, *Cacopsylla picta*-apple tree-apple proliferation phytoplasma and *C. pruni*-stone fruit-ESFY phytoplasma, regarding the behavior (oviposition, feeding) and fitness (mortality, developmental time, size) of the respective vector insect mediated by metabolites of infected and uninfected plants.

The influence of host plant infection status onto feeding behavior of the respective vectoring insect was assessed by electropenetrography (EPG). Phytoplasma infection in the apple pathosystem resulted in changes in feeding and oviposition behavior of *C. picta*, wheras no behavioral changes were recorded in the stone fruit pathosystem. Analyses of both primary (amino acids, sugars) and secondary metabolites (sesquiterpenes) in the host plants by gas chromatography coupled with mass spectrometry revealed significant differences in both apple odor and phloem composition induced by the phytoplasma, but no changes in the stone fruit. Additionally, the fitness of *C. picta* but not of *C. pruni* was negatively influenced by phytoplasma infection of their host plant. The impact of the results for the understanding of the evolution of phytoplasma-vector pathosystems and for the application in plant protection are discussed.

Keywords: *Candidatus* phytoplasma; psyllid vector; EPG; phloem composition; VOC

Insect-induced changes in fungal chemical exudate composition impact key properties of an experimental decomposer system

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Secondary metabolite biosynthesis is a pervasive and often environmentally induced process by which organisms influence ecosystem properties. Understanding the causes and consequences of these biochemical dynamics is a central challenge in ecology. Although fungi have crucial functions in ecosystems, the involvement of their secondary metabolites in affecting community members remains elusive. We present experimental evidence that insect-induced changes in the chemical composition of fungal exudates released into the environment can have community-wide consequences. Fungal exudates resulting from a complex interaction between fungal genotype and insect attack not only affected insect foraging patterns and survival, but also the population growth of key microbes in a model decomposer system. Therefore, induced changes in the composition of fungal exudates may provide a critical but hitherto overlooked link between plasticity in chemical phenotype of fungi and dynamics in ecological communities.

Fruit, flies and fungi – studies of *Drosophila suzukii* ecology for pest control

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Insects and microorganisms use fruit as a substrate for their development. We studied the ecological relation between the fruit-infesting dipteran pest, the spotted wing drosophila, *Drosophila suzukii*, yeast and mold. Behavioral assays with larval and adult *D. suzukii* showed that infestation of fruit is influenced by different factors related to fruit quality, presence of yeast or the development of mold. Interestingly, various of the factors that determine the suitability of fruit as a substrate for *D. suzukii* oviposition and development are interconnected. Understanding the ecological interactions underlying fruit infestation might help to develop tools for management of *D. suzukii*. Specifically, the association between *D. suzukii* and the yeast *Hanseniaspora uvarum* appears promising for application in pest management based on the strong attraction behavior induced by yeast odors. Based on our findings from the laboratory and field we think that formulations of the yeast *H. uvarum* could be of use for monitoring and control of *D. suzukii* in horticultural production.

Keywords: bait; egg-laying; fermentation; habitat; symbiosis

Bark beetle-associated fungi assist in the detoxification of host flavonoids and provide a fitness benefit the Eurasian spruce bark beetle

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Bark beetles are a major cause for the decline of conifer forests world-wide which are a sink for atmospheric carbon dioxide, and hence an important buffer against climate change. Bark beetles are associated with ophiostomatoid fungi which are thought to assist them to successfully complete their life cycle. The Eurasian bark beetle, *Ips typographus*, transports its associated fungi to a new host which establish in the tree when the beetle tunnels through the phloem to lay its eggs. The tree responds to beetle attack by producing resins which are defensive and can deter bark beetles from persisting in the host. However, it is not known if other host compounds also contribute to the tree's defense against *I. typographus*. Inoculations of Norway spruce saplings with the bark beetle-associated fungus, *Endoconidiophora polonica,* revealed that it responds to fungal infection by producing high concentrations of the flavonoids, catechin and taxifolin. These compounds reduced the tunneling rate and weight gain of *I. typographus* when applied *in vitro*. Furthermore, bark beetles avoided tunneling in substrates with high concentrations of catechin. However, this behavior was reversed when *E. polonica* was established on the medium first. *In vitro* growth assays of *E. polonica* revealed, that the growth of the fungus was also retarded by catechin and taxifolin, but that it could detoxify these compounds. The beetle's preference for substrates colonized by its associated fungi may indicate that fungal detoxification of flavonoids is beneficial to the beetle and contributes to its fitness during attacks on chemically well-defended hosts.

Keywords: taxifolin; catechin; *Endoconidophora polonica;* flavonoids biosynthesis; tree defence

Outsourcing immunity: Evolution, chemical ecology, and genomics of protective insect-bacteria symbioses

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Symbiotic associations with microbes are important driving forces of evolutionary innovation. Several insects engage in symbiotic alliances that protect their immature stages against pathogen infection through the production of antimicrobial compounds. I will report on novel findings of symbiont-provided antibiotic defense in beewolf wasps and darkling beetles, discussing the ecological dynamics and evolutionary implications of defensive symbiosis, as well as the genomic consequences for the bacterial partners.

Keywords: mutualism, Streptomyces, Burkholderia, antibiotic, entomopathogen.

Catabolism of branched chain and aromatic amino transferases, route to fusel alcohols and acetates by the Ceratocystidaceae.

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Fungi in the family Ceratocystidaceae produce fusel alcohols and acetates that have fruity and floral odours. Since fusel alcohols are produced in fungi from amino acids via the Ehrlich pathway in a three-step catabolic process, we identified and characterized the genes encoding enzymes involved in catalysing the first and second steps in this pathway. We identified three putative branched-chain amino transferases, three putative aromatic amino transferases and a putative pyruvate decarboxylase in each of the *Bretziella, Berkeleyomyces, Ceratocystis, Davidsoniella, Endoconidiophora, Huntiella* and *Thielaviopsis* genomes. Using gas chromatography coupled to mass spectrometry (GC-MS), also revealed that all the strains included in this study produced high levels of isoamyl acetate. In contrast, only some members of *Bretziella, Berkeleyomyces, Ceratocystis* and *Huntiella* produced isobutyl acetate, while only the *Berkeleyomyces, Ceratocystis* and *Huntiella* strains produced 2-phenylethyl acetate in low quantities. Either by accepting the branched-chain amino acid substrates (valine and leucine) or accepting the aromatic amino acid substrate (phenylalanine). Fusel alcohols and acetates produced by fungi in the Ceratocystidaceae can therefore be used as additives of food products, perfumes and soaps. In addition, identified fusel alcohols can potentially be utilized as biofuels or biodiesels, bringing solutions to the problems associated with limited fossil resources and climate change.

Keywords: Fusel alcohols; Fusel acetate; Ceratocystidaceae; Ehrlish pathway; Gas Chromatography Mass Spectrometry

Biosynthesis of aggregation pheromone component in spruce-killing bark beetle *Ips typographus*

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The main problem in the European forestry in the last decade is the epidemic population of European spruce bark beetle (*Ips typographus*, Coleoptera) causing the destruction of host habitat, Norway spruce, *Picea abies* forests. The successful aggregation of *Ips typographus* on host tree and ability to overcame the defense is triggered by the possessing of the potent aggregation pheromone. Active pheromone compounds (mainly 2-methyl-3-buten-2-ol and *cis*-verbenol) are produced by the pioneering males while invading the host tree and starting to bore in. Understanding the major precursors of the pheromone biosynthesis and molecular mechanisms over the beetle's developmental stadia is the straightforward approach to possible regulation of the aggregation behaviour. We chose a several methodological approaches to describe this process. We constructed dynamic production curve of pheromone components, intermediates and associated metabolites by the metabolomics analysis of different developmental beetle stadia by the GC/MS and UPLC/MS. We corelated transcriptomes of *Ips typographus* tissues in various life stadia using Differential Gene Expression (DGE) analysis with aim to target key genes involved in biosynthesis of main pheromone components and intermediates and we validated its regulation level by qRT-PCR.

Keywords: cis-verbenol; 2-methyl-3-buten-2-ol; European spruce bark beetle; terpene biosynthesis

ABSTRACTS:

Insect Microbe Interactions: Poster Presentations

In programme order

Poster Session 1

Stressed-induced changes in the microbial community of *Populus nigra*

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The plant microbiome can benefit plants by promoting their growth and defence against pests and diseases. The structure and function of the microbiome is very dynamic and is affected by the host genotype and the environment. Plants produce various secondary metabolites and volatiles in response to abiotic and biotic stressors that may facilitate specific interactions including with microbial partners. In this study, we investigated changes in the metabolome of poplar in response to infection by *Fusarium euwallaceae* and *Graphium euwallaceae*. The plant volatiles collected using a “pull-push” dynamic headspace collection system were analysed using gas chromatography coupled to mass spectrometry (MS). Semi-polar secondary metabolites were extracted with methanol and analysed using liquid chromatography coupled to MS. We found quantitative differences in the volatiles and secondary metabolites in plants inoculated with fungi compared to control plants. Furthermore, there were differences in the volatile composition of the plants inoculated with different fungal species. Using these results, this study aims to explore whether these fungus-induced compounds affect the bacterial community. Furthermore, we aim to determine if the bacterial community structure changes when a fungal competitor is introduced, and if there is a gradual shift in community structure from young tissue to older tissue.

Keywords: dichloromethane; GC-MS; LC-MS; metabolites; quantitative; semi-polar.

Exploring the potential nutritional symbiosis between *Ips typographus* and its fungal associates

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The European spruce bark beetle (*Ips typographus*) is one of the most infamous European forest pests. Despite this beetle's obvious relevance to the forest industry, fairly little is known about its ecology. The bark of conifer trees represents a harsh environment for insects, not only because it is fortified with layers of defenses meant to keep them out, but also because it lacks nutritional compounds to support insect development. Microbes have been found to assist insects in both these situations, either by detoxifying host defensive compounds or by supplementing insects' diets with nutrients that may be missing from or in an unusable form in the host tree. *Ips typographus* is known to be associated with fungal exosymbionts, although the role or roles these fungi play remains unclear. We hypothesized that a major role could be symbiosis through nutritional supplementation. To investigate this, we first analyzed concentrations of macronutrients in these fungi using LC-MS/MS and performed elemental analysis to determine their carbon-to-nitrogen ratios. We also used an artificial rearing system to observe the effect of fungi on bark beetles from the time of hatching to eclosion as callow adults. Results from these assays suggest that exposure to certain fungal symbionts influences bark beetle development, possibly via nutritional supplementation. Understanding the nutritional impact these symbionts may have will lead to a better understanding of bark beetle ecology and their management as a pest species.

Keywords: Coleoptera; forestry; microbe; mutualism; pest

Behavioural responses of *Rhagoletis cerasi* flies to volatiles from the yeasts populating cherry berries

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The European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae) is the most important pest of sweet and sour cherry (*Prunus avium* L. and *Prunus cerasus* L., Rosales: Rosaceae) berries in Europe. Without insecticide treatment, up to 100% of fruits can be infested. Chemical and biological control means as well as formation of mechanical barriers around trees are currently used for cherry fruit fly control. Up to our knowledge, no efficient semiochemicals have been identified for attraction of *R. cerasi* flies. The goal of this study was to identify volatiles released by yeasts colonizing cherry berries that could have behaviour modulating effect on *R. cerasi* flies. Yeasts of five species attributed to *Pichia*, *Hanseniaspora*, *Metschnikowia*, *Aureobasidium*, and *Cryptococcus* genera emitted around eighty volatiles as shown by gas chromatography-mass spectrometry analyses of samples obtained using headspace methods. A gas chromatography-electroantennographic detection analysis of yeast volatiles revealed six electrophysiological active compounds classified as esters and alcohols. Two esters and an alcohol showed the most pronounced activity in Y-tube olfactometric tests and were selected for tests in cherry orchards. In the field assay, traps baited with alcohol caught more fruit flies compare with the un-baited traps. Our results demonstrated the efficacy of yeast volatiles as baits for trapping *R. cerasi* flies.

Keywords: fruit fly; sweet cherry; sour cherry; chromatography-electroantennography; field assay

The role of the gut microbiome in overcoming the chemical defenses of *Eucalyptus* species in the *Eucalyptus* snout beetle, *Gonipterus sp. n. 2*

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The *Eucalyptus* snout beetle is an oligophagous invasive insect of *Eucalyptus* species. The beetle is attracted to emerging leaves containing high concentrations of essential oils consisting of a complex mixture of mono and sesquiterpenes. In *Eucalyptus*, terpenoids can repel insects through powerful odours and bitter taste. Furthermore, on a molecular scale, terpenoids are involved in breaking down cell membranes and affecting ion transport negatively. Regardless of the elaborate chemical defences of *Eucalyptus* species, the *Eucalyptus* snout beetle is able to tolerate and overcome varying concentrations of terpenoids. We hypothesize that the environmentally acquired gut microbial community of *Gonipterus sp. n. 2* is involved in the detoxification of *Eucalyptus* secondary metabolites. *Gonipterus* beetles were reared on two *Eucalyptus* host genotypes with significantly different biochemical profiles. The metabolomes of frass collected from reared *Gonipterus* beetles were compared with leaf metabolomes. Metabolomic analysis indicated vast differences in the metabolite profiles between the leaf material and the beetles' frass. Additionally, between the leaf and the frass profiles, terpenoid concentrations from leaf to frass dramatically decreased. Furthermore, several biotransformation products, detoxified by cytochrome p450s and dioxygenases, were identified in the frass, including numerous oxygenated and hydroxylated products of 1,8-cineole, α-pinene and limonene. These findings suggest that the gut microbiome of *Gonipterus sp. n. 2* may facilitate in the detoxification of plant secondary metabolites.

Keywords: essential oils; microbial communities; plant secondary metabolite; terpenoids