ABSTRACTS:

Recent discoveries and new approaches to the study of plant volatiles and their roles in plant communication: Oral Presentations

In programme order

Session 12 (Part I)
Session 15 (Part II)

Plant-Plant Communication via Volatiles Triggers Growth and Defense Synchronization among Plants

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The capacity of individual plants to rapidly detect and respond to "true" signals that point to real upcoming threats from neighbors is essential as it determines their growth strategy and survival. Volatile organic compounds (VOCs) realized from herbivory damaged plants are the most common signals that reveal emitter physiological status to neighbors. In our studies, we investigated whether VOCs emitted by undamaged plants may also be used as signals in the detection of competitive neighbors and whether plant response can be modified if the VOCs are changed by other types of plant interactions. We found that one undamaged barley variety exposed to VOCs of another variety allocated more biomass to the roots and become less attractive to aphids. Low red: far red (R:FR) light is signal that provides reliable information about the presence of competitors. Emitter barley exposed to R:FR allocated more biomass to above ground parts and changed the blend of VOCs released which induced the same response in exposed barely variety making it more competitive for the light-foraging, but not for defense. Touching between plants is another signal in detection of competitive neighbors. We have demonstrated that plants respond to touch by upregulation of early defense genes and by changes in the emission of VOCs that activated the same defense genes in neighboring plants. Our studies show that highly specific signals embedded in the volatile profile of emitting plants can transfer information about their changed physiological status to neighbors, triggering growth and defense synchronization among nearby plants.

Keywords: acclimation; aphids; coexistence; plant-insect interactions; plant-plant interactions

Perception and Response in Hybrid Aspen: Volatile-Induced Defence Traits

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It has been convincingly demonstrated that plants can detect and respond to volatile organic compounds (VOCs) infiltrating their environment. Sources of response-eliciting VOCs include the plant itself, neighbouring plants, and herbivores. Despite a wealth of information accumulating to document elevated resistance in VOC-receiving plants, questions remain about the degree of the receiver plant response, the scope and flexibility of VOC-encoded information, and the general ecological significance of such interactions. Using hybrid Aspen, *Populus tremula* x *tremuloides*, as a model plant, and *Phratora laticollis* as a model herbivore, we investigated volatile-mediated intra-plant, inter-plant and herbivore-plant interactions. A combination of laboratory studies and fieldwork was conducted to investigate responses of the receiver plants to VOCs from different sources. Analyses of a suite of metabolic responses provides evidence that receiver plants prime emission of VOCs and secretion of extrafloral nectar in response to cues indicative of a future herbivore presence. We will discuss these results in the context of perception of volatiles by plants and the influence of environmental complexity.

Keywords: Herbivore; plant-herbivore interactions; plant-plant interactions; priming; salicylaldehyde.

Differential defence responses of Neotropical maize genotypes to Fall Armyworm, Green Belly Stink bugs, herbivore-induced volatiles and the plant elicitor indole

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Herbivore-induced plant volatiles (HIPVs) emitted by Neotropical maize can prime indirect defence in neighbouring plants, resulting in the recruitment of natural enemies. Using a Brazilian population of *Spodoptera frugiperda*, we investigated i) the development of *S. frugiperda* larvae on five Neotropical maize genotypes (Zapalote Chico, Mirt 2A, Sintético Spodoptera L3, BRS 4103 and BRS 1040) with differing benzoxazinoid (BX) levels and ii) the effect of HIPVs and the known plant elicitor indole upon BX production. Furthermore, using the stink bug *Dichelops melacanthus*, we explored whether or not other generalist herbivores are affected by varying BX levels in maize. When feeding on the selected maize genotypes, *S. frugiperda* larvae took an additional week to pupate in genotypes BRS 1040 and Mirt 2A, but larval survival was the same and high (> 70%) on all the genotypes. When *S. frugiperda* larvae fed on BRS1040 and Mirt 2A genotypes, production of Bxs in these genotypes was suppressed, suggesting that *S. frugiperda* larvae can alter maize defence plant responses in its favour. By contrast, when the SS genotype was exposed to HIPVs followed by *S. frugiperda* larvae or indole, BX levels were higher. Survival of *S. frugiperda* larvae on indole-treated plants was significantly reduced. In contrast to the effects seen with *S. frugiperda*, *D. melacanthus* feeding enhanced the production of BXs in the SS genotype. Furthermore, survival of male and female *D. melacanthus* was also reduced by pre-exposure of the SS genotype to indole.

Key words: maize genotypes, benzoxazinoids, direct defence, fall armyworm, stink bug

Commented [CA1]: Do you mean that you exposed plants to HIPVs and then to larval feeding? Or just HIPVs? Please clarify.

Commented [MF2R1]: Yes. The plants were exposed to HIPVs for 24h and after they received S. frugiperda larvae.

Helicoverpa zea Caterpillars Manipulate Volatile Emission from Maize Through Salivary Glucose Oxidase Activity

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The glucose oxidase (GOX) effector in the saliva of many herbivorous caterpillars may induce or reduce the emission of volatile organic compounds (VOCs) from host plants. However, the ability of this effector to either induce or decrease VOC emissions appears to be largely dependent on the plant itself. Since maize-produced green leaf volatiles (GLVs) and indole prime neighboring conspecifics and herbivory-induced terpenes act as signals for parasitoids, we examined the influence of GOX on the emission of maize GLVs, indole, and terpene volatiles. Maize treated with synthetic GOX emitted a lower quantity of GLVs and higher quantities of induced terpenes compared to PBS treated control plants. A similar pattern of volatile emission was observed from maize treated with salivary gland homogenates from wild-type *Helicoverpa zea* (with high GOX activity) and CRISPR-CAS9 gene edited *H. zea* unable to produce GOX. Interestingly, the synthetic GOX treatment did not induce indole from maize, while the application salivary gland homogenate from wild-type and mutant *H. zea* induced indole emission. Mediation of volatiles by GOX is likely mediated partially through stomatal closure as has been shown in several crop systems. The ramifications for GLV emission reduction through *H. zea* GOX activity are unknown in the context of the GLV-indole priming signal to nearby maize plants. Additionally, increased terpene emission from GOX-treated maize has unknown effects on the ability of parasitoids to learn the volatile signal emitted from caterpillar-damaged maize. Further study will examine the time-course of terpene synthase transcript induction with GOX treatment.

Keywords: Glucose oxidase; *Helicoverpa zea*; insect effectors; volatiles; plant-insect interactions

Silencing the alarm: An insect salivary enzyme closes plant stomata and inhibits volatile release

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Herbivore-induced plant volatiles (HIPVs) are widely recognized as an ecologically important defensive response of plants against herbivory. Although the induction of this “cry for help” has been well-documented, only a few studies has investigated the inhibition of HIPVs by herbivores, and little is known about whether herbivores have evolved mechanisms to inhibit the release of HIPVs. To examine the role of herbivore effectors in modulating HIPVs and stomatal dynamics, we conducted series of experiments combining pharmacological, surgical, genetic (CRISPR-Cas9) and chemical (GC-MS analysis) approaches. We show that the salivary enzyme, glucose oxidase (GOX), secreted by the caterpillar *Helicoverpa zea* on leaves, causes stomatal closure in tomato (*Solanum lycopersicum*) within 5 min, and in both tomato and soybean (*Glycine max*) for at least 48 h. GOX also inhibits the emission of several HIPVs during feeding by *H. zea*, including (*Z*)-3-hexenol, (*Z*)-jasmone, and (*Z*)-3-hexenyl acetate, which are important airborne signals in plant defenses. Our findings highlight a potential adaptive strategy where an insect herbivore inhibits plant airborne defenses during feeding by exploiting the association between stomatal dynamics and HIPV emission.

Keywords: effector; HIPV; insect herbivore; plant defense; stomata

Fall Armyworm Oviposition Suppresses Volatile Emission in Maize: Effects on Recruitment of Egg Parasitoid

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Plants subjected to insect infestation often increase volatile emission to attract natural enemies, although some arthropods have evolved ways to suppress volatile profiles of host plants. Concurrently, natural enemies have developed a finely tuned olfactory system to locate plants on which their prey is present. Our earlier studies showed that egg deposition by stemborer moth (*Chilo partellus*) elicited increased levels of volatile emission from certain maize genotypes which attracted natural enemies of the pest. In this study, we investigated whether similar effects could be observed due to egg deposition by an alien invasive pest to Africa, the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and the multitrophic consequences thereof. Interestingly, *S. frugiperda* oviposition suppressed volatile emission in maize when compared to control intact plants and changed the ratio of components in the emitted blend resulting in distinct volatile profiles. In an olfactometer bioassay, the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) was significantly attracted to volatiles from egg-infested maize plants compared to uninfested controls. Our findings imply that egg deposition by *S. frugiperda* triggers an early herbivore alert signal involving suppression of maize volatile emission; however, the herbivore's key egg parasitoid is tuned in to the cue. The implications of the findings in exploiting native parasitoids for biological control of the invasive pest, *S. frugiperda*, are discussed.

Key words: Behavioural bioassay; *Telenomus remus;* biocontrol; *Spodoptera frugiperda*; oviposition-induced plant volatiles (OIPVs)

Using Plant Odours for Early Detection of Disease: A Case Study in Bananas

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Plant diseases reduce the production and quality of food, fibre and biofuel crops. Globally almost 16% of all crops are lost to disease every year. Early detection of plant disease is vital to control spread and limit impacts on horticultural industries. Recently, several studies have investigated the use of odour for detection of disease, e.g. sniffer dogs to detect citrus greening disease (huanglongbing) in Florida and a smartphone-based VOC-sensing platform for detecting late blight in tomato. Panama disease (Fusarium wilt) is the most devastating disease of bananas of modern times, caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc). When grown on rice, race 4 isolates of Foc that are infective in 'Cavendish' bananas produce distinctive odours, which are not produced by race 1 isolates to which 'Cavendish' bananas are not susceptible. We previously described how headspace volatiles from 'Lady Finger' bananas inoculated with Foc sub-tropical race 4 (STR4), were different from controls, before external symptoms were evident. The same was not true for 'Cavendish' bananas inoculated with Foc STR4. Fungal odours were detected in the corm of both varieties when they were destructively sampled. Here we describe studies on the impact of infection with the more aggressive strain of Foc (tropical race 4 - TR4), and the patterns of plant and fungal odours produced in glasshouse and field trials. Foc TR4 has rapidly spread through banana production worldwide and now threatens the major exporting countries in Central America. Early detection of the disease is critical to containment strategies.

Keywords: fungi, Fusarium wilt, GC-MS, *Musa*, volatiles

Tissue-specific volatile-mediated defense regulation in maize leaves and roots

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Herbivore induced plant volatiles (HIPVs) can mediate plant defenses in neighboring plants. Yet, whether this phenomenon occurs belowground remains unknown. Given (i) that roots can emit a distinct bouquet of volatiles upon herbivory, (ii) that some of these volatiles can diffuse in the soil, and (iii) that some root volatiles can be perceived by neighboring plants, we hypothesized that HIPV-mediated defense regulation may occur in the roots as well. We infested maize plants with the root herbivore, *Diabrotica balteata*, and investigated the physiological responses of neighboring plants. Exposure to root HIPVs did not alter constitutive nor herbivore-induced levels of defense-related gene expression, phytohormones, volatile and non-volatile primary and secondary metabolites, growth and herbivore resistance in roots. Cross-exposure experiments between roots and leaves HIPVs revealed that maize roots, in contrast to maize leaves, neither emit nor respond strongly to known defense-regulating HIPVs. Together, these results demonstrate that volatile-mediated defense regulation is restricted to the leaves of maize plants. This finding is in line with the lower diffusibility of volatiles in the soil and the availability of other, potentially more efficient information conduits belowground.

Keywords: belowground plant-herbivore interactions, maize, plant-plant interactions, priming, volatiles, root defenses

Towards Spatial Analyses of Community Composition: What Role for Plant Volatiles?

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Essential biodiversity variables (EBV's) set priorities, sampling and measurement standards for global monitoring. Only two EBVs exist for community composition: taxonomic diversity, and species interactions. We aim to develop scalable approaches for both of these using remote sensing and spatially resolved sampling. As plants are the trophic base of many communities, and sessile, we are interested in plant properties which can serve as useful indicators. Plant specialized metabolites – metabolites not required for cell growth and differentiation, which have diversified over the course of evolution – can be indicative of plant identity and condition, as well as strong determinants of ecological interactions and community structure. One example are the volatile compounds released by plants under stress. For example, a tree eaten by caterpillars will release volatiles which may be specific to the caterpillar species, and severity of attack. These volatiles can attract natural enemies of the caterpillars and activate defensive responses in neighboring trees. Laborious, time-resolved sampling and sample processing have hindered testing of community-level hypotheses. We aim to establish remote sensing and high-throughput, spatially resolved sampling approaches for metabolites of interest and lift this barrier.

Keywords: essential biodiversity variables (EBV's); plant-plant interactions; plant responses; plant specialized metabolites; species interactions

Plant-Associated CO_2 Mediates Long-Distance Host Location and Foraging Behaviour of a Root Herbivore

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Insects use different cues to locate and select suitable hosts. What are the plant metabolites that guide and are used by root herbivores to locate their host plants from a distance? There is abundant literature on the topic, which is plenty of predictions, but little direct evidence for the involvement of individual metabolites. The development of modern methods to study how root volatiles modulate foraging behaviour of root herbivores are urged to tests these predictions and provide direct evidence in this context. We developed an RNAi system to impair the perception of CO_2 in the larvae of the western corn rootworm and use it to study the importance of plant emitted CO_2 to modulate host location and larval foraging behaviour. We found that the expression of a carbon dioxide receptor, *DvvGr2*, is specifically required for larval responses to CO_2. Impairing CO_2 perception has no effect on the ability of WCR larvae to locate host plants at short distance (<9cm), but strongly impairs host location at greater distances. We also found that WCR larvae preferentially orient and prefer plants that grow in well-fertilized soils compared to plants that grow in nutrient-poor soils, a behaviour that has direct consequences for larval growth and depends on the ability of the larvae to perceive root-emitted CO_2. This study unravels how CO_2 serves as a distance-dependent host location cue and provides evidence for the power of modern genetic manipulation approaches to study plant volatiles and their roles in plant communication and ecological interactions in general.

Keywords: Plant-herbivore interactions; foraging; volatile perception; behavior; host location.

Plant Volatiles in Invasion Scenarios

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Climate change and biological invasions constitute two of the main threats to biodiversity. An essential aspect for developing sustainable management strategies and diminishing species loss is understanding the responses of organisms and ecological communities to changing environments. There is an increasing awareness of the impact of climate change on plants, including its effects on volatile organic compound (VOC) emissions and their ecological roles. However, less is known about plant VOCs in invasion settings. Interesting questions include: Do exotic plants behave chemically different in their native and invaded ranges? Which environmental factors influence the volatile emissions of these species in their invaded ranges? Do native plants respond to invaders by changing their chemical profiles? And; which are the potential impacts of these new chemical environments at the community level? This talk will use information obtained during five years of research on the invasive weed *Calluna vulgaris* (heather) in the Central Plateau of New Zealand to address some of these questions. We will also highlight knowledge gaps and propose new avenues for future research on VOCs in invasion scenarios.

Keywords: invasive species; volatile organic compounds; plant competition; plant-insect interactions, metabolomics.

ABSTRACTS:

Recent discoveries and new approaches to the study of plant volatiles and their roles in plant communication: Poster Presentations

In programme order

Poster Session 4

Direct and Indirect Defense Induction on Maize by Neighbouring Molasses Grass

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Plants have evolved intricate defence strategies against herbivore attack including mechanisms to eavesdrop on herbivore- or wound-induced volatile organic compounds (VOCs) emitted by neighbouring plants to activate direct and indirect defences. VOCs released by molasses grass, *Melinis minutiflora* (P. Beauv.) has been shown to repel stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a major pest of maize in sub-Saharan Africa, and enhance larval parasitism by *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae). However, the role of VOCs signalling in molasses grass-maize interaction and any subsequent effect on the stemborer pest and its natural enemy (parasitoid) behaviour remains unknown. This study examined the effect of plant-plant signalling on *C. partellus* oviposition and *C. sesamiae* attraction by intercropping *M. minutiflora* with locally adapted maize cultivars (landraces), Nyamula and Jowi-red. In two-choice oviposition bioassays, *C. partellus* preferred non-exposed maize landraces for egg deposition to those exposed to molasses grass. Meanwhile, the parasitic *C. sesamiae* wasp was significantly attracted to volatiles from *M. minutiflora* exposed maize compared to unexposed control in a four-way olfactometer bioassay. The coupled chromatography (GC)-mass spectrometry and GC-linked electroantennography analyses of headspace samples from maize landraces exposed to *M. minutiflora* revealed strong induction of bioactive compounds such as (*E*)-4,8-dimethyl-1,3,7-nonatriene. Our findings suggest that constitutively emitted *M. minutiflora* VOCs can induce direct and indirect defence responses in neighbouring maize plants. The plant-plant volatile signalling could offer a novel and ecologically sustainable strategies of crop protection against the destructive stemborer pest that are relevant to smallholder maize farmers in the region.

Key Words: *Chilo partellus*, induce defence, volatile organic compounds, maize landraces, *Melinis minutiflora*.