Behaviour Special Interest Group Online Meeting

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ABSTRACTS

1. INSECT CUTICULAR HYDROCARBONS AS VOLATILE SEMIOCHEMICALS

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Insect cuticular hydrocarbons are typically involatile, high molecular weight alkanes, often with one or more methyl substituents or double bonds. Their primary function is to protect the cuticle and waterproof it to prevent dessication. In several groups of insects it is well-established that they have evolved secondary rôles as contact semiochemicals. For example, in many species of cerambycid beetle they serve to distinguish sex and species and may also function as trail pheromones. More recently it has become apparent that cuticular hydrocarbons are essential components of volatile sex pheromones in at least two groups of insects, Lepidoptera and Hymenoptera. These cuticular hydrocarbon components are often of very low volatility and have very different chemistry from the more "conventional" components of the pheromone produced in the pheromone gland. They typically elicit no electroantennographic response from the insect antennae, and yet they can synergise the attractiveness of more conventional pheromone components quite dramatically. Some recent examples in moths and sawflies will be discussed in relation to their behavioural activity and chemistry and the consequent difficulties in their discovery and identification.

2. HARNESSING BENEFICIAL SOIL MICROBES FOR SUSTAINABLE AGRICULTURE AND PEST MANAGEMENT: CHALLENGES, OPPORTUNITIES, AND OUTSTANDING QUESTIONS.

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The changing climate and crop losses due to insect pests make attaining food security, an urgent global issue, a complex and challenging target. Beneficial soil microbes have tremendous potential to provide innovative and sustainable approaches to increase plant productivity and improve pest management. Numerous studies including my research have established that colonization of plants by beneficial soil microbes such as plant growth-promoting rhizobacteria and mycorrhizal fungi can increase plant growth, improve crop productivity, and induce systemic resistance against insect herbivores. Despite the documented benefits and the high potential of microbial-based solutions for agriculture and pest management, a few challenges remain. I will synthesize my findings focused on

understanding the factors that shape the outcomes of beneficial soil microbe plant and insect interactions. The talk will also highlight the challenges, opportunities, and outstanding questions. A comprehensive understanding of beneficial soil microbes-plant insect interactions can pave the way and provide novel opportunities to manipulate these interactions to promote crop yields and food security.

3. INTERSPECIFIC SIGNALS TO DETER OVIPOSITION BY SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*, SWD)

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The worldwide invasive fruit pest, *Drosophila suzukii* can lay eggs in fruit before it is harvested. Larvae degrade fruit as they feed on the pulp and the oviposition holes allow entry of fungal and bacterial pathogens. Currently fruit growers rely extensively on frequent applications of insecticides to protect fruit, but increased regulatory restrictions on pesticide usage, ecological impacts and likely emergence of insecticide resistance make this strategy unsustainable in the longer term. Cues for *D. suzukii* egg laying are likely to be a combination of visual, olfactory, and gustatory. However, it is not clear what cues deter *D. suzukii* from egg laying in ripening fruits. Recent studies by our group and other researchers have shown interactions between *D. suzukii* and other closely related *Drosophila*. We demonstrated that *D. suzukii* females are deterred from laying eggs (ovipositing) on artificial substrate or fruit where eggs have previously been laid by *D. melanogaster* (Shaw et al., 2018). This suggests that *D. suzukii* detect a chemical signal released by *D. melanogaster* which deters *D. suzukii* from egg laying. The project aims to 1) determine the origin of the oviposition deterrents to *D. suzukii*, whether the signal originates from *D. melanogaster* adults or immature stages, 2) identify the mechanism through which *D. suzukii* detect the oviposition deterrent, 3) identify the chemical compound or composition of the chemicals that deter SWD from ovipositing, and 4) laboratory and field test of oviposition deterrents for control of *D. suzukii.*

Shaw, B. et al., (2018). Pest Manag. Sci. 74 : 1466-1471

4. 'PUSHING' TOWARDS A NEW IPM STRATEGY: *DROSOPHILA SUZUKII,* THE SEARCH FOR NEW REPELLENTS

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Drosophila suzukii is a worldwide pest causing serious economic losses to the horticultural industry. It is the only *Drosophila* species in Europe possessing a serrated ovipositor, enabling females to lay eggs in ripening fruit. Adult *D. suzukii* overwinter in woodland as a low-temperature resistant winter morphs infests the crop at the start of the growing season. Reducing the influx of winter morphs into the crop could potentially reduce pest pressure for the remaining season. The aim of this project is to identify novel repellents which can protect fruit from both the winter and summer morphs of *D. suzukii*. Electrophysiological assays were conducted to identify which of a panel of fourteen potential repellents can be detected by olfactory cells on the antenna of both the winter and summer morph. Fourteen detectable compounds were then screened through behavioural trials, the results of which identified seven chemicals which were repellent to summer morphs and five which repelled winter morphs. Four of these potential repellents were formulated into release devices and tested in polytunnel trials, the aim of which was to identify those chemicals which could reduce the number of *D. suzukii* attracted to fruit held in traps, compared to a control. Three of the four chemicals tested reduced the number of *D. suzukii* caught in traps and numbers of eggs laid in sentinel fruits. Current work aims to measure the effectiveness of these repellents in preventing oviposition in strawberry crops, and to identify barriers to uptake of this new technology.

5. REPRODUCIBILITY IN BEHAVIOURAL EXPERIMENTS

W.J.Budenberg, T.A.Allen & E.Harrison

Zantiks Ltd, Cambridge, UK.

A major issue that slows down research is that many behavioural experiments can be hard to reproduce. Many factors contribute to this, and though many of these can be controlled with enough time, money and resources, these are often not available.

We present today a solution to some of these problems, with the use of a highly automated and controlled experimental setup, available at relatively low cost and of small size (the Zantiks MWP unit). We present data collected using the system from multiple locations and dates in the UK, and compare the results. The experiments focus on habituation & PPI studies of Drosophila to light off startle responses, and mosquito larvae and pupae startling to vibrations of different frequency and power.

The Zantiks MWP unit is an integrated experimental chamber which provides a controlled and isolated environment for behavioural research. Environmental (temperature and lighting) and experimental (lights, millisecond accurate stimulation, vibrations) stimuli are all integrated in the unit, and are controlled from the included computer. Video recordings and live tracking are also integrated within the experimental control system. Other external stimuli can also be controlled - e.g. odour presentation. Experiments are run using a simple scripting language, and scripts may be

shared between sites / units, so that experiments are accurately replicated. The unit is accessed via a local browser interface. Scripts also define the format of and data to be collected, and statistical analysis can be performed directly on the outputted data files.

6. THE MORE THE MERRIER? ATTRACTIVENESS OF YEAST SPECIES, ALONE AND IN COMBINATION, TO *DROSOPHILA SUZUKII* (SPOTTED WING DROSOPHILA).

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Drosophila suzukii is an economically damaging pest, of soft and stone-fruit, present in most northern temperate regions. *D. suzukii* is attracted to yeasts for feeding and overwinters as a morphologically distinct winter form. Here we tested if *D. suzukii* attraction varied, not only between yeast species, but also combinations of yeasts, and if preferences differed between summer and winter morphs.

Three separate choice tests, using a 32-channel Locomotor Activity Monitor, were done to test the attractiveness; 1) of single yeast species, 2) combinations of yeasts singly fermented then combined, and 3) co-fermented yeasts all grown in Yeast Peptone Dextrose (YPD) media to *D. suzukii* summer and winter morphs.

Activity of flies visiting the yeasts was recorded over 24-hours and counts combined into four-hour periods. All five single yeast species tested were attractive for at least one four-hour time period compared to the YPD control, for both summer and winter morph flies. Three out of four combinations, of singly fermented then combined yeasts, and both co-fermented combinations tested were attractive to both morphs. Four single yeasts and three combinations (singly fermented) were more attractive than YPD across more time periods for winter compared to summer morph *D. suzukii*.

This research demonstrates that the choice of yeast is important for the different morphs of *D. suzukii*, and that careful consideration is needed in how these might be manufactured for use in baits for attract and kill strategies. Further tests are being undertaken to investigate which yeast 'formulations' will be most effective combined with insecticides.

7. GENETICS OF BEHAVIOURAL ISOLATION IN *HELICONIUS* BUTTERFLIES

Richard Merrill, Matteo Rossi & Alexander Hausmann

Many species remain separate not because they fail to produce hybrids, but because their individuals effectively 'choose' not to mate in the first place. Although the significance of behavioural barriers has been recognized at least since the Modern Synthesis, we still know little about the genetic changes that underlie the evolution of mating preferences, or variation in

behaviours across natural populations more broadly. The warning patterns of the Neotropical butterflies *Heliconius cydno* and *H. melpomene* are under disruptive selection for mimicry, and are also used during mate recognition. We report a genome-wide QTL analysis which reveals that divergent male preference between these species has a surprisingly simple genetic basis. Three QTLs explain 60% of the difference in preference behaviour observed between the parental species. One of these QTLs is physically linked to the major wing patterning gene *optix*, which causes a switch in forewing colour from white to red. Genetic linkage between loci for ecological and assortative mating traits are predicted to facilitate speciation in the face of gene flow. By integrating these results with gene expression and population genomic analyses, we identify candidate genes responsible for shifts in visual mate preference behaviours. These candidates suggest shifts in behaviour involve changes in visual integration or processing, allowing preference evolution without altering the perception of the wider environment. Emerging data also suggest behavioural alleles may be acquired through introgression, allowing reassembly of existing genetic variation into new combinations, further facilitating the rapid evolution of novel behavioural phenotypes.

8. INSECT HOST SEEKING BEHAVIOUR: INTEGRATION OF VOLATILES ACROSS TIME AND SPACE TO MAXIMISE FITNESS

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In an environment with changing availability and quality of host plants, phytophagous insects are under selection pressure to find suitable hosts. They can improve their fitness (survival and reproductive success) by locating suitable plants and avoiding unsuitable ones. Thus, they have evolved a finely tuned sensory system, for detection of host cues, and a nervous system, capable of integrating inputs from sensory neurons with a high level of spatio-temporal resolution.

Insect responses to cues are not fixed but depend on the context in which they are perceived, such as host availability, the physiological state of the insect, and prior learning experiences. Space and time play crucial roles in influencing the outcome of interactions between insects and plants. Furthermore, interactions can be altered by other organisms associated with the plant such as other insects, plant pathogens, or mycorrhizal fungi.

Insect adaptation is not perfect, especially as hosts are a moving target. While insects have evolved ways of finding hosts, plants are under selection pressure to evade detection and defend themselves when attacked. Once on a plant, insect-associated molecules can trigger or suppress volatile emission resulting in a changed volatile blend. How induced blends impact insects depends on whether the plant or the insect is ahead in evolutionary terms.

Conversely, plant reproductive fitness is increased by attraction of pollinators and in these mutualistic interactions, the plant is under pressure to attract pollinators. There is a trade-off between attracting pollinators and repelling herbivores.

9. A NOVEL NON-STICKY TRAP DESIGN SUITABLE FOR THE MONITORING OF SOME *AGRILUS* JEWEL BEETLES INCUDING THE INVASIVE *A. PLANIPENNIS* (COLEOPTERA, BUPRESTIDAE) IN EUROPE

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A light, easy to handle and maintain non-sticky trap type could facilitate monitoring and detection of the Asian emerald ash borer *Agrilus planipennis* Fairmaire (EAB) and other jewel beetle populations. Sticky material-free jewel beetle catches are highly advantageous because there is no need to clean them with chemicals before determining to species. We carried out a series of trapping experiments in an ash (*Fraxinus pennsylvanica* Marshall) forest belt in Western Russia and an oak forest (*Quercus petraea* [Matt.] Liebl.) at Budapest, Hungary to test our latest experimental trap designs, which possess the above features of a non-sticky trap.

Our results suggest that the light green MULTz trap design may be suitable for catching a multitude of buprestid species in Europe, including the ash-related *A. planipennis* and *A. convexicollis* Redtenbacher, and some of the European oak-related *Agrilus* species (*A. obscuricollis* Kiesenwetter, *A. graminis* Kiesenwetter, *A. angustulus* (Illiger), and *A. laticornis* (Illiger)).

The 12-funnel Lindgren trap is also an efficient non-sticky trap design for catching EAB, while MULTz traps catch sizeable numbers of several *Agrilus* jewel beetles including EAB, in a direct comparisons of the two trap types. MULTz trap has the advantage of easier handling at a much lower estimated cost. MULTz trap is also suitable to catch live jewel beetles, ensuring specimen quality.

Field work of YB, DD and LS was supported by RFBR (grant 17-04-01486).

10. PLANT VOLATILE ORGANIC COMPOUNDS THAT AFFECT THE SEXUAL BEHAVIOUR OF *ANASTREPHA FRATERCULUS* MALES

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Plant secondary metabolites affect the sexual behaviour and sexual communication of phytophagous insects in various ways: acting as cues to "rendezvous" sites for mating; stimulating signalling behaviour; or making pheromones more attractive to the opposite sex. Here, we evaluated the effect of phytochemicals produced by guava fruit on the mating behaviour and reproductive biology of *Anastrepha fraterculus*, considering the age and nutritional status of the flies. Wild as well as laboratory males were exposed to the aroma of guava fruit and then evaluated in terms of mating success, calling behaviour, chemical signalling. Exposed males achieved significantly more matings and mated for longer periods of time than non-exposed males, irrespective to male origin. Guava exposure increased the signalling rate by 30-40% (wing fanning and salivary gland exposure) both for laboratory and wild flies. Enhanced signalling was accompanied by larger amounts of sex pheromone being released by exposed males, with no effect on the chemical profile of the male cuticle. Females mated with guava exposed males showed higher fecundity than females mated to non-exposed males, while fertility did not differ. Interestingly, the effect of guava on male sexual behaviour depended on the age and nutritional status of males. In sum, exposure to guava enhanced male mating success and signalling rate in *A. fraterculus*. The effect does not seem to be mediated by a change in the aroma of the male cuticle or an accelerated sexual maturation. Females would obtain a direct benefit from choosing exposed males in terms of realized fecundity.

Key words: insect-plant interactions, phytochemicals, sexual behaviour, aromatherapy.

11. A POTENTIAL SEX PHEROMONE ANTAGONIST FOR THE ALFALFA PLANT BUG, *ADELPHOCORIS LINEOLATUS* (HEMIPTERA: MIRIDAE)

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The alfalfa plant bug, *Adelphocoris lineolatus* (Goeze, 1778) is a pestiferous species with a wide range of host plants. Previous studies reported attraction of the species to plant-derived volatiles, furthermore, sex pheromone components have also been identified in Eastern-Asian populations of the species. Nevertheless, in case of species with such a wide area, composition of pheromone blends may differ in distant geographic regions as it was found in case of some other species.
In our studies, we performed air entrainments from alfalfa plant bugs collected from Central European populations and compounds eliciting electroantennographic activity were tested in field experiments in Hungary, to gather information on chemical ecology of Central European populations of the species.
In the course of the studies hexyl-butyrate, (*E*)-2-hexenyl-butyrate and (*E*)-4-oxo-2-hexenal were identified from the samples, these results were in concert with previous data on Eastern-Asian populations of the species. The blend showed activity in the field. Furthermore, another compound elicited conclusive electroantennographic activity, this compound was identified as 1-hexanol.

Interestingly, when tested in combination with the pheromone blend, 1-hexanol significantly decreased attraction of males to the pheromone blend. This antagonism showed dose-response effect, with relatively small doses showing remarkable effect as well. The results suggest, that this compound may act as a sex pheromone antagonist for *A. lineolatus*. Prospects for practical applications will be discussed.

12. SEMIOCHEMICAL-BASED MANAGEMENT OF THE COTTON BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOH. (COLEOPTERA: CURCULIONIDAE)

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The boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae), is the main pest on cotton crops in the Neotropical region, destroying cotton squares and bolls. Due to the high reproductive rate of adults and endophytic behavior of the immature stages, infestation levels can increase rapidly. Farmers have adopted heavy spraying of insecticides to control *A. grandis* and its aggregation

pheromone is used for population monitoring. However, when cotton reaches the reproductive stage, there is a decline in *A. grandis* response to pheromone traps, suggesting that plant attractants as well as the natural pheromone-producing weevils in the crop play a role in *A. grandis* colonization in cotton. This presentation will focus on the identification of cotton volatile kairomones responsible for *A. grandis* attraction in a series of laboratory and field experiments to understand cotton-boll weevil chemical ecology in order to optimize trapping efficiency using semiochemical-based pest management.

13. A PUSH-PULL SYSTEM TO MANAGE *ALPHITOBIUS DIAPERINUS* POPULATIONS USING THEIR TRUSTFUL CHEMICAL SIGNALS

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The lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), is the most important insect pest affecting poultry production worldwide, with all life stages being able to transmit pathogens such as bacteria, virus and fungi, which can compromise bird's health and the productivity. Control of *A. diaperinus* in poultry houses is performed by intensive insecticide application. However, this technique is not effective due to the cryptic behaviour of this pest. In this work, we evaluated the potential of recently identified *A. diaperinus* alarm (1,4-benzoquinone, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone) and aggregation [(*R*)-limonene, 2-nonanone, (*E*)-ocimene, (*S*)-linalool, (*R*)-daucene and (*E*,*E*)-α-farnesene] pheromones as tools for the management of this pest in poultry houses in Brazil. Previous studies confirmed *A. diaperinus* alarm pheromone repellence and aggregation pheromone attractiveness. In a field assay, that compared a pull (aggregation pheromone) and a push–pull system (simultaneous alarm/aggregation pheromone deployment), a higher number of *A. diaperinus* were captured in aggregation pheromone-baited traps in the push–pull system. Our results suggest that the alarm pheromone can displace *A. diaperinus* from its hiding places and allows more insects to be captured in the aggregation pheromone-baited traps. The push-

pull system with pheromones, a sustainable strategy, can be deployed in poultry houses to trap significant numbers of adult *A. diaperinus*. Studies are underway to determine the potential for using these components as part of an integrated *A. diaperinus* management.

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