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BOOK OF ABSTRACTS

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Pesticide resistance facilitates adaptation and survival through a range of different mechanisms that reduce or diminish the effectiveness of an active ingredient, for example by preventing its mode of action at the target site. Resistance to pyrethroid insecticides detected in 2014 in *Sitobion avenae* (Fabricius) (Hempitera: Aphididae), a prolific cereal pest and vector of Barley Yellow Dwarf Virus (BYDV), presents a serious challenge to cereal growers. Resistance has been attributed to the classic target site mutation (L1014F) known as Knock Down Resistance (kdr), found on one allele of the aphid sodium channel gene. Resistance screening and microsatellite genotyping over a three year period in Ireland reveals that kdr-heterozygote aphids occur extensively in cereal fields and adjoining grass verges, with resistance predominantly in one SA3 superclone. Furthermore, a second resistance mechanism in the form of pesticide detoxification has been detected in some SA3 clones, likely linked to enhanced cytochrome P450 monooxygenase activity. Resistance mutations are frequently believed to carry fitness penalties. However, our research provides evidence that whilst the heterozygote kdr SA3 *S. avenae* superclone can survive pyrethroid exposure up to twice the normal field application rate, it continues to be able to reproduce asexually at rates comparable to fully susceptible individuals. Furthermore, under laboratory conditions sexual capacity is retained in the SA3 superclone, with the observation of oviparous morphs, creating the possibility of gene-flow through a wide range of (androcyclic, holocyclic and intermediate) breeding systems, and creation of kdr-homozygote offspring through sexual crossing between kdr-heterozygotes if heterozygote resistant male aphids are produced. These findings have serious implications for resistance management and the future efficacy of pyrethroid insecticides in controlling grain aphids and the associated risk of BYDV transmission in crops.

Keywords: Fitness penalty, grain aphid, knock down resistance, pesticide resistance, pyrethroid detoxification, resistance gene-flow, SA3 superclone, sexual reproduction, *Sitobion avenae*

Insecticide Resistance: Detection & Monitoring

CO154

THE RELATIVE CONTRIBUTION OF TARGET-SITE MUTATIONS IN COMPLEX ACARICIDE RESISTANT PHENOTYPES IN *TETRANYCHUS URTICAE*

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The molecular mechanisms underlying insecticide and acaricide resistance in insects and mites are often complex. Both target-site insensitivity and an increased metabolism, transport and excretion (or a combination thereof) can all contribute to the resistance levels observed in field populations. The relative contribution of target-site resistance mutations to the resistance phenotype has remained poorly understood in most arthropod pests. Here, we used marker-assisted backcrossing to create 34 congenic lines of the a polyphagous arthropod pest, the spider mite *Tetranychus urticae*. These lines share a common pesticide-susceptible genomic background, except for 10 loci that carry mutations (alone, or in combination in a few cases) that are associated with resistance to avermectins, pyrethroids, mite growth inhibitors, mitochondrial complex I (METI-I) and complex III (QoI) inhibitors. Toxicity tests revealed that mutations in the voltage-gated sodium channel, chitin synthase I, PSST subunit of the respiratory complex I and cytochrome b confer moderate to high levels of resistance and, when fixed in a population, these mutations alone can result in field failure of acaricide treatment. In contrast, the glutamate-gated chloride channels mutations do not lead to the high resistance levels that are often reported in abamectin resistant strains of *T. urticae*. Last, we used a complementary approach to evaluate the effect of a resistance mutation in Drosophila as a model organisms. We used the CRISPR-Cas9 genome editing tool to introduce the mutation conferring resistance to METI-I in the Drosophila PSST homologue. Overall, this study functionally validates reported target-site resistance mutations in *T. urticae*, by uncoupling them from additional mechanisms, allowing to finally investigate the strength of the conferred phenotype *in vivo*.

Keywords: Near-isogenic lines, NIL, target-site mutation, *Tetranychus urticae*, backcrossing 20 kDa subunit, METI-I, rotenone, Acari, Drosophila

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USING CRISPR TO INVESTIGATE MECHANISMS OF NEONICOTINOID RESISTANCE IN *DROSOPHILA MELANOGASTER*

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Neurotoxic insecticides enter the insect body and encounter metabolic enzymes (*e.g.* cytochrome P450s and glutathione-s-transferases) during a journey that takes them to receptors in the brain. Movements between tissues are controlled by transporters, such as the ABC transporters. Appropriate mutations in genes encoding any of the proteins that interact with an insecticide could confer resistance. The potential for such resistances to evolve in the field will depend on many factors including the ratio of resistance conferred to fitness costs incurred. In our lab we have adopted a systems approach to understand the interaction between the neonicotinoid imidacloprid and each of these types of proteins. This has involved using CRISPR and controlled tissue-specific overexpression to manipulate genes, twin ion mass spectrometry to monitor levels of the insecticide and metabolites and a movement assay (the Wiggle Index) to assess the level of intoxication.

Keywords: Imidacloprid, nicotinic acetylcholinesterases, cytochrome P450s, glutathione-s-transferases, ABC transporters

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MINING GENES INVOLVED IN INDOXACARB RESISTANCE OF *LOBESIA BOTRANA* (DENIS AND SCHIFFERMÜLLER) BY *DE NOVO* TRANSCRIPTOME ASSEMBLY AND DIFFERENTIAL EXPRESSION ANALYSIS

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Lobesia botrana (Denis and Schiffermüller) (Lepidoptera: Tortricidae) is one of the most important grapevine pests in Europe but, being a non-model organism, only limited genomic and transcriptomic resources are available for functional studies at the molecular level, such as those relevant to insecticide resistance and pest control. Hence, to gain insight into the mechanism of indoxacarb resistance, a blocker of insect voltage-gated sodium channels (NaV), we analysed the transcriptome and expression profile in 2nd instars of *L. botrana* from susceptible and field selected populations (LC50 resistance ratio 72). *De novo* transcriptome assembly using Trinity resulted in 141,581 isoforms clustered in 94,290 putative genes. The transcriptome completeness was supported by BUSCO: 92% of conserved orthologs (n=1,658) were retrieved as a complete sequence, 6.3% displayed fragmented ORFs, and only 1.7% were missing. 36,250 genes were preliminary annotated relaying on the longest isoform per gene, by running Annocript pipeline against non-redundant protein databases (Nr), gene ontology (GO), cluster of orthologous groups of proteins (COG), KEGG orthology (KO) and long non-coding RNAs (lncRNAs). Conditional Reciprocal Best BLAST analysis of protein isoforms performed on Lepidoptera proteomes identified putative orthologs of multigene family members potentially involved in metabolic resistance (61 cytochrome P450 monooxygenases, 25 glutathione S-transferases, 13 carboxylesterases, 25 UDP-glucuronosyltransferases) as well as alternatively spliced isoforms of the NaV gene. Among 263 upregulated and annotated genes in the resistant population, functional GO enrichment analysis revealed overrepresentation of terms for cytochrome P450, due to up-regulation of CYP6B and CYP9A subfamily members as well as increased transcript level for UGT genes. Hydrolases were, on the contrary, overrepresented in 293 annotated genes, downregulated in the resistant population. These data tentatively suggest the reduced susceptibility to indoxacarb might be related to an increase of Phase I and II detoxification along with reduced bioactivation of the insecticide.

Keywords: *Lobesia botrana*, insecticide resistance, indoxacarb, transcriptome, differential expression analysis

CO157

MECHANISMS OF RESISTANCE TO DELTAMETHRIN IN ISOFEMALE LINES OF THE MOSQUITO *AEDES AEGYPTI* FROM FRENCH GUIANA

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Aedes aegypti is vector of dengue, chikungunya and Zika viruses in urban area of French Guiana, a French territory in South America. Deltamethrin, a pyrethroid insecticide, remains the sole insecticide molecules authorized for adult control in the European Union to which French Guiana belongs. However, resistance to deltamethrin has been monitored in several populations of *Aedes aegypti* from French Guiana and revealed an extreme resistance to this molecule. In order to better understand the mechanisms of resistance, four isofemale strains have been successfully isolated locally, with similar genetic backgrounds but with different resistance spectra to deltamethrin (pyrethroid). We performed enzymatic activity assays of detoxification enzymes involved in insecticide resistance. We also monitored mutations located at the position 1016 and 1534 of the sodium voltage-gated channel gene in these populations by Taqman Allelic Discrimination Assays. These mutations were already linked to pyrethroid resistance in *Aedes aegypti* populations from Latin America. Resistance to other insecticide families (organophosphates and carbamates) was also monitored in our strains that exhibited different level of resistance to deltamethrin. Our study revealed different combinations of resistance mechanisms in our lab strains: metabolic resistance or resistance associated to a mutation of the sodium voltage-gated channel gene. The metabolic resistant strains have an increase esterase activity while other strains exhibit mutation at the position 1016 of the sodium voltage-gated channel gene. Moreover, selection to deltamethrin led to an increase resistance to other class of insecticides suggesting inherited predisposition to multiple resistance. The results obtained on these laboratory lines are essential for understanding the resistance to deltamethrin as well as multiple resistance phenomenon in the natural populations of *Ae. aegypti* of French Guiana.

Keywords: *Aedes aegypti*, isofemale strains, insecticide resistance, multiple resistance

CO158

INSECTICIDE RESISTANCE IN UK PESTS: THE GOOD, THE BAD AND THE UGLY

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The evolution and spread of insecticide resistance in agricultural and horticultural pests continues to threaten our ability to protect our crops and imposes increased selection pressures on the active compounds that remain in our armoury. European Legislation, making restrictions on the use of neonicotinoids as seed treatments, is only exacerbating this situation. As a result, continued monitoring of any changes in the resistance profile of insect pest populations is crucial if we are to communicate relevant, up-to-date information to agronomists, growers and government regulators on which insecticides will currently be effective and which will not. This presentation will summarise the insecticide resistance profiles currently seen in a range of important UK pests (aphids, beetles, moths and thrips); information gained through work collaboratively funded by Agrochemical Companies, Commodity Boards and Agronomy Companies.

Keywords: Insecticide resistance, insect pests, insecticides

CO159

WESTERN CORN ROOTWORM RESISTANCE TO BT MAIZE WITHIN AGRICULTURAL LANDSCAPES

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The western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is a serious pest of maize in the United States and is currently managed by planting transgenic maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt). In some portions of the United States, western corn rootworm has evolved widespread resistance to transgenic maize producing Bt toxin Cry3Bb1. Resistance to Cry3Bb1