Abstracts
of oral
presentations

Innovative approaches to sustainable pest management in UK agriculture

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In order to support food security, farmers need to have access to a wide array of tools to manage pests, weeds and diseases. To minimise the harms from pesticides, we are encouraging farmers and growers to work holistically and use a diverse set of integrated pest management strategies, which will mean that chemical control can still be used but in a more targeted manner, enabling the chemistry to remain effective for longer before resistance develops. At Defra, we understand the multiple challenges that farmers face and the importance of cutting-edge scientific research in understanding the risks from pesticide resistance and new pest pressures and in developing the tools to manage these effectively.

Putting resistance management into action: An industry perspective

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Resistance management needs to be based in excellent research but ultimately it needs to be implemented by farmers across the globe. Farmers need to buy into resistance management, with a belief in why it is needed and a clear understanding of how it can be implemented.

CropLife International is an association of multinational companies involved in research and development in plant sciences. It represents a global structure and member companies for whom resistance management is a key tool for sustainable crop protection with both pesticides and plant biotechnology. CropLife has been promoting a systems approach to resistance management, in partnership with key organisations.

A key pillar of CropLife's strategic approach has been the inclusion of Mode of Action (MoA) icons and information on pesticide labels. These simplify the choice of pesticides for rotations or mixes for resistance management. Globally there has been a significant growth in MoA labelling and countries with mandatory MoA labelling requirements. Stewardship has been supporting this label information through considering key influences on farmer behaviour, including the cost and benefits of implementing resistance management and behavioural insights that should make outreach more effective.

Organophosphate resistance in Myzus persicae: exploring new discoveries in the genomic era

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Over 30 years ago the molecular mechanisms of organophosphate tolerance in peach potato aphid Myzus persicae, became one of the best-studied examples of insecticide resistance in a crop pest. A series of publications, many of which came from Rothamsted Research, identified both the target site polymorphism and a metabolic component involving two closely related carboxylesterase genes called E4 and FE4. Subsequent molecular characterization at the time pinpointed the mechanism of resistance to an increase in gene copy number and changes in methylation for either E4 or FE4. However, the specific mechanism(s) by which these genes were amplified was never elucidated. The evolution of whole genome and transcriptome sequencing technologies created an opportunity to revisit this old story and fill the knowledge gaps which could not be addressed at the time. Thanks to access to over 100 live aphid clones with various levels of esterase resistance, we have combined the latest advances in long-read DNA sequencing, whole genome methylation calling and copy number estimation to gain a better understanding of how the esterase resistance emerged and is regulated in *Myzus persicae*.

The K^+/Cl^- Cotransporter Represents a Putative Target to Reduce Resistance to Dieldrin (rdl)

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The γ-aminobutyric acid (GABA) receptor and GABA-receptor-chloride-channel (GRCC) complex is an established insecticide target, yet target site mutations in *rdl,* the gene encoding GABA-R, threaten the utility of GABAergic insecticides. We defined the functional interplay between the $GABA_A$ receptor and K^+/Cl^- cotransporter (KCC) as a putative mechanism to reduce rdl phenotypes. KCC concomitantly extrude K^+ and Cl^- from the neuron to produce low intracellular Cl^- levels and enable inhibitory synaptic signaling through inward flux of Cl^- . Our data show that CNS-specific ablation or pharmacological inhibition of *Drosophila* KCC increased CNS spike discharge frequency and reduced GABA potency by 3-fold. Further, KCC inhibitors at an EC_{10} increased sensitivity of the *Drosophila* CNS to dieldrin by 4-fold that verified functional coupling of KCC to GRCC. Co-treatment of a KCC inhibitor (EC_{10}) and dieldrin increased the CNS firing rate by 70% in flies carrying *rdl* mutations but did not alter the firing rate in susceptible flies, which suggests KCC inhibition may rescue rdl phenotypes. Indeed, toxicity bioassays with rdl-carrying *Drosophila* showed co-treatment of dieldrin with KCC inhibitors reduced the resistance ratio to 7-fold compared to 90-fold with dieldrin alone. These data indicate KCC represents a putative target to mitigate resistance phenotypes to GABAergics.

The delay in confirming QoI-resistance in *Puccinia horiana* in Japan: overlooking risks beyond the G143A and F129L mutations

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Resistance to QoI fungicides, conferred by target site mutations, is widely recognized among various pathogen populations. The major mechanisms of resistance are the G143A mutation in highly resistant strains and the F129L mutation in moderately resistant strains. Although the G137R mutation plays a minor role, it is frequently mentioned in the literature. In Japan, chrysanthemums account for 40% of ornamental plant production. White rust, caused by *Puccinia horiana*, has consistently been the most problematic pathogen for growers. Despite their efforts to adopt an IPM approach, the application of fungicides remains necessary, and the number of spraying is quite high. As a result, this species has become a hotspot for fungicide resistance among crop pathogens in the country. By around 2010, several years after the initial use, some farmers had already reported poor efficacy and suspected QoI resistance. Nevertheless, researchers could not confirm these suspicions because they did not detect the G143A, F129L, or G137R mutations and faced difficulties in obtaining reference-sensitive isolates of this obligate parasite. In 2019, resistance mediated by several mutations, initially identified in phytopathogens, was confirmed. This story, along with a cautionary note for fungicide researchers, will be presented.

Counteract Herbicide Resistance through Herbicide Selectivity: A Case Study on Selective Herbicide Safening in *Arabidopsis thaliana*

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There are more than 300 weed species that confirmed to develop resistance to multiple herbicides. To enable the diversification of herbicides to counteract resistance weeds, the herbicide selectivity in crops is a crucial factor. Safeners have long been used to facilitate herbicide selectivity by enhancing the expression of detoxifying enzymes such as glutathione transferases (GSTs) in monocot crops. Even though safeners are known to induce the expression of detoxification genes in dicots, safeners do not protect dicot crops from herbicide toxicity. The aim of this study is to understand the cause of safener failure to protect dicot species which could then lead to the development of effective dicot safeners. Using the model dicot *Arabidopsis thaliana* and the safener isoxadifen-ethyl (IDF) as a test system, seedlings and root cultures were treated with IDF to test for any enhancement in GST-mediated metabolism of the herbicides flufenacet and *S*-metolachlor. The combined analysis of transcript expression, herbicide metabolisms, enzymatic reactions and phenotyping of T-DNA insertion *Arabidopsis* mutant plants confirmed the importance roles of GSTU7 in the detoxification and safening toward flufenacet in *Arabidopsis.* Our study demonstrates that safening effect in dicot can be achieved in the more restrict manners than those in monocot species.

"Exploring the Resilience and Adaptability of Agricultural Weeds"

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Among the various traits exhibited by weeds, resilience and adaptability enable weeds in the agri-environment to withstand or rapidly adapt to both human-imposed control methods and environmental challenges such as abiotic or biotic stresses. Despite their importance, our understanding of the molecular mechanisms underlying either of these traits remains limited. Our research aims to address this knowledge gap through physiological, molecular, and genomic analyses of blackgrass as well as other globally problematic weeds. We quantify and compare the physiological traits and transcriptomic or metabolic pathways that give weeds their competitive edge over the crops they infest. We functionally validate interesting targets including by transiently manipulating gene expression *in planta*. We undertake comparative genomics using genomes we have sequenced, both the Mendelian-inherited chromosomes and extra-chromosomal circular DNA. Together, these approaches give us a detailed understanding of the mechanism(s) weeds use to withstand the challenges of the agri-environment. Through our molecular studies in agricultural weeds, we aim to both pinpoint vulnerabilities that can be targets for sustainable weed management and uncover the secrets of weed's resilience and adaptability, thus providing valuable insights for safeguarding sympatric crops against future challenges, including from the weeds themselves.

Multi-drug resistance in the fungal wheat pathogen *Zymoseptoria tritici*: a hint to complex mechanisms

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The agricultural sector faces increasing development of fungicide resistance in *Zymoseptoria tritici*, the agent of septoria leaf blotch in wheat. Since the early 2010s, strains with 'Multi-Drug Resistance' (MDR) phenotype have been detected in natural populations. This phenotype is associated with the overexpression of the membrane efflux pump gene *MFS1* due to three distinct promoter inserts, resulting in increased efflux of fungicides. A *Z. tritici* population survey for MDR strains in 2020/21 led to the isolation of new MDR strains whose resistance profiles differ from previous field isolates and transgenic constructs indicating that additional mechanisms to *MFS1* promoter inserts may be at play.

Experimental evolution studies have also led to the selection of MDR strains unlinked to *MFS1* overexpression. To elucidate whether increased efflux is involved in these phenotypes, efflux tests were carried out on a selection of MDR isolates. The majority of the studied isolates exhibited an increase in comparison to the ancestor strain suggesting that their MDR phenotype may be attributed to increased fungicide efflux. Other isolates showed different phenotypes, indicating the involvement of alternative MDR mechanisms. Whole-genome sequencing revealed unknown genes potentially involved MDR. The involvement of a previously uncharacterized transcription factor in MDR will be presented.

Genetics of insect resistance to Vip3Aa

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The VIP (vegetative insecticidal proteins) toxins from *Bacillus thuringiensis* kill insects via different pathways compared with the Cry toxins. Vip3Aa is deployed in transgenic crops as a backup against pest evolution of Cry toxin resistance, and is currently effective against the fall armyworm, *Spodoptera frugiperda*. However, Vip3Aa-resistant strains of several pest species are now known. Two different mutations associated with resistance to Vip3Aa have been reported from lab-selected strains of invasive *S. frugiperda* from China. One is a change in the promoter sequence of SfMyb, a transcription factor that apparently controls the expression of protein binding targets of Vip3A in the midgut. Another is insertion of a transposable element that disrupts the coding sequence of SfCHS2, the chitin synthase responsible for chitin production in the peritrophic matrix in the midgut. Knockouts of the chitin synthase gene conferred resistance to Vip3Aa in *S. frugiperda* and two other lepidopteran pests. We expect that mutations conferring *S. frugiperda* resistance to Vip3Aa occur in loci other than *SfMyb* and *SfCHS2*. Anticipating and managing Vip3Aa resistance is important for sustainable pest control, especially with field-evolved resistance to Cry toxins conferred by a dominant mutation affecting tetraspanin and associated with increased protease activity.

A new player involved in metabolic resistance in *Echinochloa crus-galli*

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Metabolic resistance is the common mechanism of herbicide resistance in grass weeds. However, the molecular mechanism behind this resistance remains poorly understood. This study aimed to elucidate the resistance mechanism of a *Echinochloa crus-galli* var. *formosensis* line (Ecf108) that exhibits resistance specifically to an acetyl-CoA carboxylase (ACCase) inhibiting herbicide cyhalofop-butyl. LC-MS/MS analysis revealed an enhanced metabolism of cyhalofop-acid in Ecf108. Two rounds of RNA-seq analyses were conducted using multiple *E. crus-galli* lines collected from various regions in Japan, identifying 62 highly expressed genes in Ecf108. Among the candidate genes, *EcCBR1* conferred resistance to cyhalofop-butyl in rice calli. Since *CBR1* exists as two copies in diploid grass genomes, we used CRISPR/Cas9 to individually knock out the homologs of *EcCBR1* in rice. The resulting mutants showed increased sensitivity to cyhalofop-butyl, indicating the crucial role of these genes in plant metabolism of cyhalofop-butyl. Further investigations are underway to explore the involvement of *EcCBR1* in the evolution of cyhalofop-butyl resistance in *E. crus-galli*.

Searching for new “biofungicides” based on oligonucleotides for the control of fungal diseases

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Botrytis cinerea and *Podosphaera xanthii*, the causal agents of the gray mold and the cucurbit powdery mildew diseases, respectively, are limiting factors of horticultural crops production worldwide. Their control is very dependent on the application of commercial fungicides; however, both have developed high levels of resistance to the most chemical classes, a fact that has been widely demonstrated in Spain. In addition to this problem, the recent European Green Deal will be reduced the diversity of fungicides available to growers by 50% in 2030. For this reason, alternative control tools and molecules with fungicide activity are needed. In our research group, the efficacy of the emerging RNA interference (RNAi) strategy, called "spray-induced gene silencing" (SIGS), and the use of aptamers have been checked as sustainable solutions to be used into the integrated management programs of both diseases. For this purpose, several double-stranded RNA (dsRNAs) and two aptamers, involved in the virulence/pathogenicity of both pathogens, have been designed. To improve their applications under field conditions, their nanoencapsulation have also be carried out. The progress made in our research works will be presented.

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Sub-lethal insecticide stress alters epimutation rate and transposable element expression, but not mutation rate, in *Myzus persicae*

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A long-standing hypothesis proposes that exposure to low doses of pesticides may increase the mutation rate in pest genomes and consequently accelerate the evolution of resistant phenotypes. Yet the impact of pesticide-induced stress on the mutation rate of insects has not been empirically tested. We ran a two year long mutation accumulation experiment using the global pest aphid *Myzus persicae* to interrogate spontaneous genetic and epigenetic mutation rates in insecticide-exposed and unexposed aphid lines. Our data reveal that long-term exposure to sub-lethal concentrations of the neonicotinoid insecticide imidacloprid over 50 generations does not increase genetic mutation rate. Instead we find that imidacloprid exposure results in a significantly lower epimutation rate and enhances the transcriptional activity of transposable elements, as well as inducing the downregulation of numerous genes related to neuronal function and development. Our findings challenge the proposed link between pesticide exposure and the rate of mutation and suggest that a more nuanced view of this hypothesis is required.

Tiny tech, big impact: Long read sequencing in the fight against fungicide resistance

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Uncovering the molecular mechanisms of fungicide resistance along with rapid, simple and scalable detection and monitoring programmes are essential to keep up with the rapid pace of resistance evolution. We used the Oxford Nanopore Technologies MinION DNA sequencer to characterise a resistance mechanism involving copy number changes of the demethylase inhibitor target gene *Cyp51A* in the barley pathogen *Pyrenophora teres* f. *teres* (*Ptt*). Our analysis revealed a long repetitive tandem sequence in which *Cyp51A* copies were found in alternation with transposable elements. These tandem sequences also contained predicted effector-like genes that are currently being characterised. Furthermore, we developed a simple and effective monitoring pipeline to detect both known and new mutations in *Cyp51A* in *Ptt*, *P. teres* f. *maculata* and a hybrid of these two forms, with SNP level accuracy. Our approach detects both known and novel mutations in both the promoter and coding sequence of *Cyp51A*, increasing the simplicity, accuracy and ease of resistance detection. Long read sequencing has enabled new insights into the molecular mechanisms of fungicide resistance in *P. teres* and effective ways of monitoring its evolution.

Identification of a single glutathione-*S*-transferase gene associated with atrazine resistance in *Amaranthus palmeri*

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Atrazine is an important herbicide for controlling *Amaranthus* species in corn-soybean production systems in the Americas. Excessive use of the herbicide, however, has resulted in resistance evolution in a number of *A. palmeri* populations. Analysis of the *psb*A gene has shown that resistance to atrazine in the *A. palmeri* populations is not due to a target-site mutation. Here, we have carried out an RNA-seq experiment to determine the gene(s) involved in non-target-site-based resistance (NTSR) to atrazine. Whole transcriptome data from nine sensitive and ten atrazine-resistant US populations identified a single Phi-class glutathione-*S*-transferase gene (denoted ApG*S*TF1) that was constitutively over-expressed several hundredfold in the resistant as compared to the sensitive populations. Heterologous expression of ApGSTF1 in *E. coli*/tobacco further confirmed the involvement of the enzyme in atrazine resistance. ApGSTF1 also conferred resistance to the chlorotriazine herbicides terbutylazine and cyanazine but not to the other photosystemII herbicides tested. This study identified the first fully validated gene that endows non-target-site resistance to triazine herbicides in the increasingly problematic *Amaranthus palmeri* species. Development and implementation of an Elisa-specific assay will determine the prevalence of ApGSTF1 in a large number of *A. palmeri* samples from Mid-Western USA.

Sublethal exposure to pesticides and transgenerational plasticity in insect pest *Leptinotarsa decemlineata*

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While the resistance models often highlight genetic mutations at the target site as the cause of resistance, recent evidence suggests that polygenic resistance involving different behavioral and physiological adaptations, may better explain regional differences in insecticide resistance. For example, despite a similar frequency of resistance-associated alleles in the *Ldace2* gene the Colorado potato beetles (*Leptinotarsa decemlineata*) from Vermont, USA are 8-16 times more resistant against insecticides than the beetles from Belchow, Poland. Vermont population achieve this resistance through additional amino acid replacements in another *Ldace1* gene, increased *Ldace1* gene expression, and reduced sensitive to acetylcholinesterase (AChE) inhibition by insecticides. In addition to strong directional selection by lethal dosages of insecticides, polygenic resistance can also be selected by mild sublethal insecticide or other pesticide exposure. This is because mild stress is known to affect the whole genome and many genes at the same time, thus selecting for overall performance. Sublethal insecticide exposure can have further transgenerational effects on fitness-related traits (survival and body mass). These findings highlight the complexity of resistance as it is a result of selection, genetic architecture, and stress tolerance. Understanding these processes is, however, crucial for effective pest management and sustainable agriculture.

Multiple Resistance of Important Oomycetes - An increasing threat for Resistance Management in times of Limited Chemical Options

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Plasmopara viticola and *Phytophthora infestans* represent the most devastating grapevine and potato diseases caused by Oomycetes. Consequently, grapevine downy mildew and potato late blight control mostly relies on compounds being developed to specifically inhibit Oomycota species. Important active ingredients belong to the compound group of phenylamides, benzamides, CAAs, QiIs, QioSIs, carbamates, cyanoacetamide-oximes, and OSBPIs. For sound disease- and resistance management, todays´ spray schedules usually include a broad diversity of the above mentioned chemical control options, in-line with FRAC use guidelines. However, according to recent FRAC reports [1], resistance cases have been meanwhile reported for the majority of effective oomyceticides, and latest sensitivity monitoring often show occurrence of isolates bearing resistance to multiple active ingredients and biochemical mode of action. Increasing knowledge in molecular biology and genome sequences of plant pathogens has enabled development of new detection and quantification methods for resistance, adding valuable insights to data from standard sensitivity test systems. Recent examples from resistance research with *P. viticola* and *P. infestans* are presented, outlining a lack of specific control solutions in certain grapevine and potato growing regions and the need for more, innovative chemical options with new mode of action.

[1] Anonymous, www.frac.info, 2024

Target-site resistance diagnosis reimagined: a new high-throughput method and its application to analyze hundreds of weed populations

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Herbicide resistance in weeds is threatening crop yields worldwide. Molecular techniques help to diagnose target-site resistance (TSR), a main mechanism for herbicide resistance. However, current methods are costly, slow, and lack scalability, limiting full assessment of resistance in weed populations. Also, because of high false negative rates, low TSR levels may go undetected. We present a cost-efficient, highly scalable method that combines sample pooling with compact and affordable Nanopore sequencing. We investigated the quantitative and qualitative distribution of TSR in hundreds of *Alopecurus myosuroides* (blackgrass) populations in Germany and *Eleusine indica* (goosegrass) populations in Mauritius. To estimate how TSR frequencies change in *A. myosuroides* after a single herbicide treatment in real-world conditions, we collaborated with German farmers to sample before and after spring application. Furthermore, we present the method's power to assess homeologue-specific TSR allele frequencies in polyploids and a workflow to detect low TSR in seed samples of commercial *Lolium* spp., which could represent a possible source of dispersal of resistant weedy *Lolium* spp. As herbicide resistance management becomes more complex, high-resolution diagnosis becomes increasingly important. We are confident that our new method can make significant contributions to addressing herbicide resistance in both research and the crop value chain.

Genetic architecture of pesticide resistance in the mite, *Halotydeus destructor*, from its invasive Australian range

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The redlegged earth mite, *Halotydeus destructor* (Tucker 1925: Trombidiformes, Penthaleidae), is a major pest of Australia's grain and pasture crop industries. Native to South Africa, this mite invaded Australia in the 1920s and was managed very successfully with chemical pesticides for many decades. However, since the early 2000s, resistance to organophosphate and pyrethroid pesticides has increased and spread throughout Australia, making it increasingly difficult to control this pest. Genomic approaches and a chromosome-level reference assembly have been paramount in characterising the genetic architecture of pesticide resistance and the dynamics of resistance evolution in *H. destructor*. We show that populations from across Australia can evolve resistance by combining target-site mutations, copy number variation, and other genetic mechanisms. Moreover, population genomic analyses suggest that resistance often evolves independently in local populations and is unlikely to be spread via dispersal. These findings have contributed to increased awareness that local strategies matter and the importance of farm-level management. Our findings have also been instrumental in developing molecular diagnostic tools that will facilitate resistance monitoring in this invasive mite pest.

Quantifying selection on and origins of resistance mutations through space and time

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The evolution of resistance in response to herbicides is a striking example of repeated, human-induced evolution. Nonetheless, the evolutionary genetic mechanisms underlying their origins and rate of spread have been largely unquantified over spatial and temporal scales. Towards this goal, we have been studying one of the most problematic weeds in the Midwestern US, common waterhemp. Combining paired natural-agricultural sampling from the present day with sequences from historical specimens, we demonstrate the remarkable rate of resistance evolution over the last two centuries, even in comparison to newly pinpointed agriculturally adaptive alleles. Over the same period, I show concurrent shifts in population size and structure—highlighting a strong link between demographic and selective contemporary change. I will then discuss how this rate of evolution is in part driven by multiple origins of resistance, regardless of the complexity of mutations, from single nucleotide polymorphisms to structural variants. These approaches can help diagnose the spatiotemporal scales at which resistance management should occur across complex landscapes

Updating the principles of resistance management: a new theoretical framework

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When building a resistance management plan, it is useful to work with clear and distinct evolutionary mechanisms for how resistance can be delayed. Resistance modelling in the 1980s was helpful in defining those mechanisms, which were influentially reviewed in the following decade to build a consensus on the principles of resistance management. Recent resistance modelling has challenged the consensus, bringing new approaches and considerations to resistance management. This work has also led to recent changes in resistance management policy (e.g. IRAC). Here, building on old and new publications, we provide a synthetic review of the evolutionary mechanisms that delay resistance. We propose an updated classification of the principles of resistance management. We discuss how these principles can complement and clash with one another in alternative strategies, including some are based on new approaches to resistance management. Overall, we show how these principles provide a rational framework for explaining how to design a resistance management plan to delay resistance evolution.

Adaptation of *Ramularia collo-cygni* towards SDHIs and DMIs

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Ramularia leaf spot caused by *Ramularia collo-cygni* is a significant disease in barley, classified as a high-risk pathogen by the Fungicide Resistance Action Committee (FRAC). QoIs have been highly effective for Ramularia control, however resistance developed soon after market introduction with a rapid spread across Europe. Other groups of fungicides, such as SDHIs and DMIs have been also affected by adaptation to these modes of actions. The molecular mechanisms have been elucidated and the evolution of these and their effects on sensitivity loss have been intensively studied. There are several mutations causing SDH resistance with different levels of resistance. Mutations causing the strongest resistance are less frequent (H153R, G171D) and those causing low (N87S) to moderate adaptation (H146R) are dominating with regional differences. Although the outstanding efficacy of SDHIs may be diminished, they are still valuable tools for disease control and resistance management. Additionally, various CYP51-haplotypes have been identified which cause low or moderate adaptation towards DMIs. However, there is still significant disease control, e.g. by the recently introduced DMI - mefentrifluconazole. Most recent data on the evolution of SDH- and CYP51-haplotypes in Europe and effects of control strategies on disease control and mutation selection will be presented.

Characterization of multiple herbicide resistant populations of *Amaranthus palmeri* from Europe

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Amaranthus palmeri ranks among the most problematic weed species in American crop fields and is now spreading across Europe. With resistant populations to ALS- and EPSPS-inhibitors already described in Spain, suspicion increasing regarding the presence of multiple resistance to both modes of action in local populations. Moreover, the evolutionary events leading to these herbicide resistances remain unknown. In dose-response experiments with glyphosate on two roadside Spanish populations, a high survival rate was observed at a dose of 400 g a.e. ha-1. These same populations also exhibit high resistance to ALS-inhibitors thifensulfuron-methyl, and low resistance to imazamox. Target-site resistance appears to be the most prominent mechanism in both treatments. Plants surviving glyphosate showed increased EPSPS copy number variations but no mutations in the target protein. Surprisingly, survivors of ALS inhibitor exhibited 9 different mutant alleles in codons: Pro-197, Asp-376, and Trp-574. Given the history of selection pressure and the high incidence of detected mutations, we suspect that these populations have evolved resistance to both MoA in the country of origin. Further study on the eccDNA sequence in both Spanish populations seems to reinforce this idea. The presence of *A. palmeri* populations with multiple resistance poses a high risk European crops production.

Keywords: Amaranthus palmeri, herbicide resistance, ALS, EPSPS, eccDNA.

Predicting fungicide resistance by experimental evolution

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An important area of progress in resistance research in recent decades has been a gradual shift from reactive studies to a more pro-active approach. This includes monitoring for early shifts in sensitivity before resistance reaches a level where growers experience control failures, and the requirement for resistance risk assessments for new products so management guidelines can be adjusted accordingly.

Since fungicide resistance often arises from *de novo* mutations that would not be found through baseline screening, fungicide risk assessments often use artificial selection and mutagenesis. These studies can give a useful indication of overall resistance risk, but cannot always predict which of a range of mutations generated *in vitro* will emerge as significant resistant genotypes in the field. Furthermore, the predictability of resistance evolution in different pathogens in the field has varied greatly between different fungicide classes. In some cases, one or two key mutations have recurred across tens of pathogen species, whereas in others, a wide range of mutations has been reported between and within species. I am using experimental evolution to compare the evolutionary repeatability of resistance to different fungicide classes, and the role of different fungicide use scenarios in driving parallel or divergent evolutionary trajectories.

The evolutionary journey of herbicide resistant *Alopecurus myosuroides* from wild to weed

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As a key weediness trait, herbicide resistance presents a unique opportunity to understand weediness evolution and the effects of strong anthropogenic selective pressures in rapid adaptation. In Europe, blackgrass (*Alopecurus myosuroides*) has become a major threat to crop yields, mainly due to the recent and rapid evolution of herbicide resistance. Here, we aimed at reconstructing its spread in European agricultural landscapes, to gain insight into its evolution from wild to weed. Exploiting a comprehensive collection of 65 wild and weedy populations, we traced blackgrass history in space and time: we conclusively pinpointed its origin to the Black Sea region and followed its spread across Europe, making use of both contemporary individuals and pre-Green Revolution germplasm. We showed that modern weedy European populations are genetically differentiated from native and past populations. Moreover, we shed light into the genetic basis of herbicide resistance in blackgrass, defined by an oligogenic architecture, and characterized by loci under positive selection. This distinctive system helps us to delve into fundamental questions in evolutionary quantitative genetics, providing insights into the genetic basis of adaptation to novel environments and into the evolutionary processes underpinning plant populations responses to strong anthropogenic pressures.

Development and Application of a Coupled Population Dynamics and Population Genetics Mathematical Model to Link Insecticide Selection, Fitness Costs and Vector Control.

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Insecticides, deployed as insecticide-treated nets and indoor residual sprays, are the primary tool available for the control of mosquito-borne diseases, especially malaria. There is a need to develop and evaluate insecticide resistance management (IRM) strategies to mitigate the impact insecticide resistance (IR) has on disease control. We present a coupled mosquito population dynamics and population genetics model, allowing for the simultaneous evaluation of both the ability of IRM strategies to manage IR but also to control mosquito populations. The model is structured to include the mosquito life-stage, sex, genetics, behaviours, and the mating status of the female mosquito. Fitness costs are included by impacting life-history traits (egg batch size, developmental times, natural survival and mating success). We present simulations which consider both insecticide selection during vector control and the relaxation of selection pressure as may occur when insecticides are rotated out. The detrimental effects of IR on disease control are accelerated by selection on male mosquitoes, but are offset by maintaining high coverage of interventions. Fitness costs affect the frequency of IR over time when the insecticide selection is relaxed but depend on RS genotype fitness and the initial IR frequency. These are important considerations when planning insecticide rotations.

Droplet digital PCR: a new molecular approach for the detection and quantification of SDHI mutations in *Stemphylium vesicarium*

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Stemphylium vesicarium is the causal agent of several plant diseases as well as brown spot of pear (BSP), which is one of the most economically important fungal diseases in European pear-production areas. Different classes of fungicides to control BSP are available, among them we find Succinate Dehydrogenase Inhibitors (SDHIs) that are considered by the FRAC from medium to high risk of resistance. In this study, we set up a sensitive and accurate droplet digital PCR (ddPCR) protocol able to detect and quantify two mutations involved in the resistance of SDHIs, early discovered in our laboratory. For the first time, the determination of allele percentage and the frequency of a mutation involved in BSP was carried out by employing ddPCR. This molecular approach provides a rapid diagnostic tool able to detect very low substitution percentages, which is very useful for fungicide resistance detection at early stages, to better apply management strategies for the contrast of BSP disease.

Monitoring and management of herbicide resistance in Denmark

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There are widespread problems with herbicide resistance in Denmark, especially for grass weeds such as *Lolium multiflorum* and *Alopecurus myosuroides*, but also for several broadleaved weed species. A semi-randomised baseline study monitoring for resistance among eleven weed species with known resistance or under suspicion for evolving resistance were conducted in 2013-15. The survey was conducted in the light of a restructuring of the pesticide tax that significantly changed the price relations between herbicides with herbicide resistance prone and non-prone modes of action. The general knowledge on herbicide use pattern in Denmark had a clear relationship with herbicide resistance among key weed species. Five weed species where resistance was widespread were included in a follow-up study in 2021. *Poa annua* was added to the list of weed species in the 2021 study as several cases of resistance had been identified in the intervening years. Herbicide resistance is considered a major treat to crop productivity by the farming community and several projects involving farmers are ongoing to showcase diverse management strategies. The involvement of farmers and demonstration of integrated weed management are instrumental to support changes in cropping systems, thus preventing, and mitigating the development of resistance.

Update on Insecticide Resistance in UK Pests: are we all doomed?

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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. In addition, European Legislation, for example, making widespread restrictions on the use of neonicotinoids as seed treatments on all outdoor crops, is only exacerbating this situation. As a result, continued monitoring of any changes in the sensitivity/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 work and, importantly, which will not. This presentation will give a 'whistle stop tour' summarising the insecticide resistance profiles currently seen in a range of important UK pests (primarily aphids and beetles); information gained through work collaboratively funded by Agrochemical Companies, Commodity Boards, Agronomy Companies and Defra.

Management drives regional level spatial distribution of demethylase inhibitor fungicide resistance in *Pyrenophora teres* f. *teres*: insight from neighbour network analysis

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Fungicide resistance in foliar fungal pathogens of annual crops is a growing global issue for crop protection. Broadscale empirical studies of the distribution of resistance can inform resistance management approach. *Pyrenophora teres* f. *teres*, the causal agent of net form net blotch disease on barley, which has developed a mutation of the *Cyp51A* allele (in the F489L position) that confers resistance to demethylase inhibitor fungicides, was used as a model system. The spatial distribution of resistance and covariate analysis were explored in a survey spanning 300 km^2 over a two-year period and captured 367 random, mixed population samples from 31 barley fields. Processed samples were analysed using digital PCR to determine the ratio of mutant to wild-type *Cyp51A* alleles, as frequency. We show the spatial distribution of a quantified frequency of fungicide resistance and present the first analyses of spatial dependency for fungicide resistance in a foliar fungal pathogen. Spatial network analyses of survey data identify host cultivar as a major factor affecting fungicide resistance distribution pattern. The identification of cultivar as a key driver of landscape fungicide resistance distribution provides the opportunity for tactical management choices to minimise spread.

Living Lab to promote innovation in weed management in Italian agricultural crops

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The use of a collaborative approach among all interested stakeholders to encourage technological innovation within farms is the basis of the so-called Living Labs (LLs). In the frame of the National Center Agritech, Spoke 4, task 4.2.1 aims to create networks of researchers, farmers and stakeholders working together to develop and disseminate innovative practices for enhancing farms' resilience in the climate change scenario. CNR research unit focuses on weed control with the aim to combine sustainability and effectiveness, decreasing the use of chemicals, preserving water, and keeping the carbon cycle balanced. The CNR-LL includes several farms distributed in North-eastern Italy, a national working group (GIRE, Italian Herbicide Resistance Working Group) and an agricultural consultancy company (Agrin). Two main approaches will be proposed to the farmers: 1) innovative management strategies addressing two topics, "water topic" will compare broadcast herbicide application and the band herbicide application and "carbon topic" will compare different managements for cover crop termination and cash crop sowing; 2) innovative agricultural machinery for weed control (e.g. weed wiper bars, weed trimmers, tools for the removal of weed inflorescences and/or seeds destruction). This should favor the implementation of alternative management strategies through a collaborative approach to increase sustainability and environmental protection.

Fungicide resistance stewardship: opportunities to identify and drive best practice

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The holistic practices encompassed in integrated pest management (IPM) align well with the principals of fungicide resistance stewardship. Using crop management techniques which delay, prevent or avoid disease from occurring reduce reliance on fungicides. Within crop protection programmes, reduced usage of individual actives either through reduced dose or application number, or through the insertion of alternatives such as elicitors or biological control agents can further reduce selection pressure. However, despite convincing evidence that reduced reliance on fungicides can reduce the risk of resistance, the science is complex and there are still mixed messages on best practice, such that less than suboptimal practices persist. Recent research shows that IPM uptake amongst farmers increases with familiarity, and also increases where they have access to independent advice and research data. Large arable farms tend to score higher for uptake of IPM practices than smaller enterprises, which perhaps reflects a greater ability to attend to open days and knowledge exchange events. The gap between higher and lower scoring farms represents an opportunity to drive the uptake of stewardship practices. Groups such as FRAG UK which gather and interpret resistance information and reach consensus views on best practice have an important role in this.

How we best manage resistance? A case study with glyphosate and paraquat resistant *Lolium rigidum*

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In Australia, *Lolium rigidum* has evolved herbicide resistance to 12 different modes of action and the management of multiple resistant ryegrass is a costly challenge for grain growers. Herbicides are sustainably used to remove exceptionally adaptable *L. rigidum* populations to protect grain crop yields in mechanized no-till systems but weed resistance evolution is an unceasing side effect. From 2022 six ryegrass populations with multiple resistance to glyphosate and paraquat (and clethodim) were detected in Western Australia. Following the initial resistance characterization, two years of intense field research occurred at different locations (three field sites) and in parallel studies under controlled conditions were conducted across those three populations with dual resistance to glyphosate and paraquat with the clear aim to find cost-effective herbicide solutions. The study documents a novel type of multiple resistance occurring in broadacre settings, initially selected on marginal areas (fencelines) and then spreading into the adjacent cropped paddock. This study demonstrates that a science-based choice and subsequent deployment of herbicide mixtures is an essential strategy to defeat weed resistance. Our thought-provoking study suggests there is no resistance that can't be managed by herbicide mixtures and useful insights are drawn towards herbicide efficacy stewardship and cost-effective weed resistance management.

Characterization of a multi-resistant field population of *Plutella xylostella* from Taiwan

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The diamondback moth, *Plutella xylostella*, poses a significant global threat to crucifer crops and has become notoriously challenging to control due to widespread insecticide resistance. This study characterizes a field-derived strain of *P. xylostella* from Taiwan, assessing its resistance to various insecticides and genotyping known target site resistance mutations. The results reveal alarming levels of resistance, with the moth showing high levels of resistance to a broad range of commercial insecticides. However, the strain remained susceptible to isocycloseram (Plinazolin®). Genotyping results indicated high frequencies (over 70%) of pyrethroid and cyclodiene organochloride resistance-related mutations, moderate frequencies (40-50%) for acetylcholinesterase inhibitors and benzoylureas resistance mutations, and low frequencies (20%) for diamide and abamectin resistance mutations. These findings highlight the prevalence of multiple resistance, necessitating careful consideration in the selection of insecticides for managing *P. xylostella* in Taiwan and potentially offering insights for global pest management strategies. Going forward, integrated pest management approaches and the development of novel insecticides are crucial for addressing this pressing issue.

Integrated Approach for Potato Late Blight Resistance Management and Stewardship

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In recent years, Europe has witnessed emergence of new strains of Phytophthora infestans exhibiting resistance to key fungicides, including fluazinam, CAA, and oxathiapiprolin. These resistances, combined with a lack of robust innovative solutions, represent a significant challenge to potato cultivation across the continent, threatening yield stability and necessitating urgent action in resistance management and stewardship strategies. An integrated approach combining field monitoring, laboratory studies, and predictive modelling to enhance late blight resistance stewardship is possible with collaboration between stakeholders. Field monitoring is employed to track the prevalence and distribution of different pathogen strains, providing crucial data for resistance management. Laboratory studies provide some valuable information to experiment the spread of resistance strains among other strains within the population of Phytophthora infestans. Furthermore, predictive modelling techniques are utilized to simulate the evolution of pathogen strains, allowing for proactive adjustments in management strategies. Recommendations for agronomic practices and crop protection products can be tailored based on the findings, emphasizing the importance of integrated pest management approaches. Overall, this comprehensive approach can offer insights into sustainable late blight resistance management, ensuring the longevity and efficacy of control measures while promoting environmental stewardship in potato cultivation.

The WHO's "Global Plan for Insecticide Resistance Management in Malaria Vectors"

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This talk will describe some interesting aspects of the WHO's "Global Plan for Insecticide Resistance Management in Malaria Vectors" (the GPIRM). First, the circumstances and events justifying the policy will be outlined, including the scaling-up of effective malaria vector control in Africa, and the resulting impact on the malaria burden. Then the processes of consensus-building and policy development will be explained. In terms of implementation, the policy has had some success in influencing the policies and practices of national malaria control programmes, and the roles of institutions such as the Global Fund and the President's Malaria Initiative will be considered. The talk will end with a discussion of the future of insecticidal malaria vector control, and its role in malaria elimination in Africa.

Which resistance management strategies work against concurrent evolution of resistance to fungicides?

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Resistance management tactics to slow selection for target-site resistance to a single fungicidal mode of action (MoA) are well-supported by modelling and experimental evidence. These tactics include minimising the dose and number of applications, and applying in mixture with a different MoA. The best strategies for managing resistance evolving 'concurrently' to two or more MoA at the same time are less clear: this situation introduces complex trade-offs. For crop pathogens such as *Zymoseptoria tritici*, multiple fungicide applications per year are often required to keep infection below damaging levels of severity. Use of mixtures may therefore require splitting the total dose of a fungicide across two or more applications, reducing the dose of each MoA per application but increasing the exposure time of the pathogen to the fungicide. We modelled the effect of dose-splitting on selection for fungicide resistance to a single MoA and found that it varies between metabolic and target-site resistance, and with fungicide efficacy and decay rates. We then explored concurrent evolution of resistance, and identified when it is better to mix two MoA (with dose-splitting) and when it is better to alternate.

Testing insecticide resistance management strategies

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Insecticide resistance management (IRM) guidance advocates that 1) alternation of modes of action (MoA) is the most effective IRM strategy, 2) insecticides should be used at the full label dose, and 3) mixtures of insecticides should include both mixture components at the doses used when applied solo. Mechanistic modelling of target-site resistance shows that, for most scenarios relevant to a wide range of crop pest species, 1) mixtures are likely to result in longer effective lives for insecticide MoAs than alternation, 2) resistance selection is minimised by using the lowest dose at which robust control can be obtained, and 3) adjusting the dose of mixture components to achieve the same combined efficacy as a solo product is a better IRM strategy. Changes to longstanding guidance require empirical proof. This paper describes cage experiments carried out to test the model findings. *Myzus persicae* populations comprising four clones, with different combinations of two target-site resistance mechanisms, were tested with several IRM strategies. Changes in resistance frequency and population control over time were quantified. The presence of the clone resistant to both MoA negated IRM strategies. In the absence of that clone, there was some evidence for faster selection with the 'double-dose' mixture.

Herbicide resistance stewardship. Contribution of mechanistic and random forest models to support diagnostic.

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Weeds are one main threat impacting worldwide the production and the quality of food, feed, and fibers. Agronomy practices are essential for weed control, but chemical control remains necessary. Repetitive treatments of herbicides, especially when a similar mode of action is used, lead to the evolution of resistance. This is a particular concern due to the fact that very few new chemical families are brought to the market. In addition weeds can evolve several resistance mechanisms based either on herbicide target modifications (Target Site Resistance), or non-target modifications (Non-Target Site Resistance). Herbicides resistance diagnostics either in greenhouse or in the laboratory is time consuming, and costly. Prediction tools to evaluate the risk of resistance evolution will greatly help to choose the best IWM strategy adapted to the local field situation. These comprise classical simulation models combining population dynamics and genetics and, recently, artificial intelligence (AI) methods such as random forest. In that report both approaches are combined with the introduction of a non-target site resistance evolution in the mechanistic model. To this end, artificial data were generated by a mechanistic model and used as training data set for a random forest classifier. Field history information was obtained by interviewing farm managers. The data includes the field histories and resistance status of *A. myosuroides* and *Lolium* ssp. of 98 fields from Germany and 131 fields from France. With accuracies of about 80% the results obtained by the random forest method applied to model generated data and real field data respectively are well comparable. In addition the simulation model revealed the existence of a tipping point beyond which sensitivity cannot be restored. This is of particular interest in defining strategies to mitigate resistance evolution. Artificial intelligence predictions can be established without knowing the resistance mechanisms but needs significant number of field data to be accurate enough. Advantages and disadvantages of both approaches will be discussed also in term of friendly use for the customers, in particular farmers.

Key words: herbicide resistance, modelling, simulation models, artificial intelligence, grasses

Posters

POSTERS

Submitting authors are underlined – for affiliations see abstracts

**** indicates a poster with a 2-minute talk on Day 1**

FUNGICIDES

1. **Pathogenicity and fungicide sensitivity of *Sclerotinia* spp. isolates associated with Sclerotinia stem rot in oilseed rape in Germany.** B. Berger and N. Zamani-Noor

2. **Sensitivity evaluation and efficacy of various fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation.** B. Berger and N. Zamani-Noor

3. **High-throughput sequencing approach to detect and quantify alleles involved in resistance to sterol demethylation inhibitors in *Cercospora beticola.*** S. Fontaine, L. Caddoux, P. Lucas, Y. Blanchard and B. Barrès

4. **Exploring fungicide resistance in multiple fungal pathogens that infect plants, animals and/or humans.** A. Cuzick, J. Seager, M. Piovesana, J. Luo, M. Urban, N. Hawkins and K. E. Hammond-Kosack

5. **Seasonal resistance shifts to pyraclostrobin in populations of *Cercospora beticola* collected from commercial sugar beet fields in the northcentral USA.** G.A. Secor and V.V. Rivera

6. **Twenty-five years of fungicide resistance research in *Alternaria solani* in the USA.** J.S. Pasche, S. Shrestha and N.C. Gudmestad

7. **Multi-year monitoring of oxathiapiprolin sensitivity in *Phytophthora infestans* affecting tomato in Italy**. I.M. Nanni, I. Oggiano, J.L. Genet, M. Mboup, V. Bosco, F. Bove, R. Bugiani and M. Collina

8. **Cost-benefit analysis of policy options for combatting anti-fungal/fungicide resistance in the clinic and in agriculture.** R. Oliver

9. **Disease severity and conidial production of QoIs-resistant *Pyricularia oryzae Triticum* isolate on wheat plants treated with Azoxystrobin**. A.F. Dorigan, R.L. Alves, P. Ricardino da Silveira, G. Alves Ramos and E. Alves

10. **Assessing quinone outside inhibitor (QoI) sensitivity of *Corynespora cassiicola* isolates.** R. Zaia, T. Faske, F. Medeiros, J. Rupe and A. Rojas

11. **Assessment of sensitivity and fitness of *Alternaria alternata* tangerine pathotype isolates to triazoles, strobilurines and carboxamide.** I.C. Lopes Pinheiro, L. Guerra-Guimarães, P. Ricardino da Silveira, S. Intra Moreira, M. L. Vilela de Resende and E. Alves

HERBICIDES

12. **** ALS herbicide resistance in tetraploid *Stellaria media*.** D. Rissel and L. Ulber

13. **** Expanding virus-mediated reverse genetic techniques to *Lolium* spp. using BSMV vectors.** M. McGroary, G.R. Littlejohn and D. MacGregor

14. **** Early detection of allelic variants endowing acetolactate synthase resistance in four Amaranthus species through an allele specific loop mediated isothermal amplification.** A. Milani, S. Di Vita, S. Panozzo and L. Scarabel

15. **** Herbicide resistance investigation in the invasive *Eragrostis plana*.** F.P. Lamego, D. Zabala-Pardo, J.R. Nachtigall, C. O. Langer and E. K. Klumb

16. **** First case in Europe of triple resistance to glyphosate, auxins and ALS-inhibitors in a population of *Bassia scoparia*.** G. Mora, J.M. Montull, J.M. Llenes, A. Cirujeda, A. Marí and J. Torra

17. **** Herbicidal dsRNA discovery by using virus-induced gene silencing (VIGS) in weeds.** A. Milani, N.Di Vita, F. Ottaviani, L. Moffa, L. Nerva, W. Chitarra, L. Scarabel, S. Varotto and S. Panozzo

18. ****Phenotyping of two different 574 allelic variants in *Amaranthus hybridus* ALS inhibitors resistant plants.** A. Milani, A. Petrozza, C. Pornaro, S. Summerer, L. Scarabel, A. Daccache and S. Panozzo

19. **Acetolactate synthase (ALS) resistance, is it a problem in the sugar beet crop?** L. DeVere, K. Hebditch, D. Brown, L. Tatnell, S.K. Cook and P.A. Chambers

20. **Herbicide-resistant *Amaranthus retroflexus* found in herbicide-tolerant crops.** P. Kosnarová, S. Bhattacharya, K. Hamouzová, M.K. Sen and J. Soukup

21. ***Lolium and Festulolium*: a growing concern as herbicide-resistant weeds.** K. Hamouzová, P. Košnarová and J. Soukup

22. **Controlling non-target site resistant *Poa annua* with novel modes of action.** J.T. Brosnan, J. J. Vargas, L. Smith, M. Mahey and E. L. Patterson

23. **Syngenta glasshouse-based testing platform for developing herbicides that can control evolved resistance in weeds.** A Cullen, W.J. Plumb, B. Campos, J. Sheridan, M Watkins and S.S. Kaundun

INSECTICIDES

24. **** The potential of using natural synergists in integrated pest management of cabbage stem flea beetle (*Psylliodes chrysocephala*).** P.A. Ortega-Ramos, G.D. Moores and S.M. Cook

25. **** An experimental-theoretical approach to understand and predict pesticide resistance evolution in *C. elegans.*** L. Q. Li, L. Parts, P. Madgwick, K. King, A. Flemming and A. Woollard

26. **How a science-driven marketing-forward approach is addressing an evolving crisis.** S. Markell, G. Tylka, G. Bird, A. Tenuta, C. Bradley, D. Mangel, H. Lopez-Nicora and T. Watson

27. **Teasing apart the contributions of the different mechanisms involved in *Myzus persicae* resistance to neonicotinoid.** C. Mottet, L. Caddoux, S. Fontaine, C. Plantamp, C. Bass and B. Barrès

28. **Mosquito ABC transporter modulation and strategies to improve insecticide delivery.** T.D. Anderson, H.M. Hernandez, X.X. Ng, and L.C. Rault

29. **Unravelling the genetic architecture of flubendiamide resistance in *Helicoverpa armigera* (Hübner) (*Lepidoptera: Noctuidae*).** D. Amado, E.L. Koch, E.M.G. Cordeiro, W. Assunção Araújo, A.A. Franco Garcia, D.G. Heckel, G. Montejo-Kovacevich, H.L. North, A.S. Corrêa, C.D. Jiggins and C. Omoto

30. **The overexpression of cytochrome P450 genes is associated with flonicamid resistance in the green peach aphid, *Myzus persicae*.** B. Zeng, B.J. Troczka, A. Pym, C. Zimmer and C. Bass

POSTER WITH DEMONSTRATION

31. **EPPO Database on resistance cases to plant protection products.** J. Martinez Perez and V. Lucchesi

Poster 1

Pathogenicity and fungicide sensitivity of *Sclerotinia* spp. isolates associated with Sclerotinia stem rot in oilseed rape in Germany

Beatrice Berger and Nazanin Zamani-Noor

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Sclerotinia sclerotiorum causes Sclerotinia stem rot (SSR) in several Brassica species. Recently, the related pathogen *S. subarctica* has been reported on Brassica, causing symptoms identical to those caused by *S. sclerotiorum*. The significance of *S. subarctica* to infecting economically important crops such as oilseed rape (*B. napus*) remains to be elucidated. Furthermore, the virulence of the pathogen and its susceptibility to fungicides remain unclear. In an ongoing study, Sclerotinia-infected oilseed rape plants were collected across Germany and *Sclerotinia* spp. were isolated. To identify whether *S. subarctica* is present among the isolates, the rRNA ITS region was amplified and sequenced. In parallel, the virulence and pathogenicity of the isolates were evaluated through their inoculation into five oilseed rape cultivars at the cotyledon stage. Of these, 17 had low (lesion area below 10%), 40 had moderate (lesion area between 10% and 30%), and seven showed high virulence (lesion area above 30%). Furthermore, all isolates were evaluated under *in vitro* conditions for their sensitivity against different groups of fungicides including azoxystrobin, boscalid, fludioxonil, mefentrifluconazole, metconazole and prothioconazole. The mycelial growth was measured and EC50 values were determined. The preliminary results demonstrated a significant difference in inhibitory efficacy among fungicides and among the isolates.

Poster 2

Sensitivity evaluation and efficacy of various fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation

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Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is an important disease of oilseed rape that can result in significant yield losses. In the current study, we have evaluated the efficacy of different groups of fungicides as well as a biopesticide, including azoxystrobin, boscalid, fludioxonil, prothioconazole, tebuconazole, azoxystrobin/tebuconazole, boscalid/pyraclostrobin, prothioconazole/fluopyram, and *Bacillus amyloliquefaciens*, on the baseline sensitivity of *S. sclerotiorum* isolates under in vitro conditions as well as the control of SSR in the field. Artificial inoculation and mist irrigation were used to induce reproducible SSR infection in oilseed rape cultivation. All compounds significantly reduced SSR severity by 36.7% to 86.9% and increased yield by 55.2% to 98.7%, TGW by 1.5% to 7.0%, oil content by 1.5% to 5.9%, and protein content by 0.1% to 1.3%. Fludioxonil, boscalid/pyraclostrobin, and fluopyram/prothioconazole exhibited the strongest fungicidal activity against SSR. The biopesticide provided 36% disease control. Under in vitro conditions, *B. amyloliquefaciens* not only inhibited mycelial growth but also the formation of sclerotia in all concentrations. Boscalid and fludioxonil exhibited the highest level of efficacy against *S. sclerotiorum*, with mean EC50 values of 1.23 and 1.60 µg a.s./mL, respectively. The highest variability of EC50 values between isolates was observed for prothioconazole and azoxystrobin.

Poster 3

High-throughput sequencing approach to detect and quantify alleles involved in resistance to sterol demethylation inhibitors in *Cercospora beticola*

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Cercospora leaf spot, caused by the plant pathogen *Cercospora beticola*, is one of the main foliar diseases affecting sugar beet. Triazoles, fungicides from the DMI group, are a key family used to control this fungus. They inhibit sterol synthesis by acting on cytochrome P450-dependent sterol 14-α-demethylase. Loss of sensitivity to these fungicides has been reported in several countries. In France, bioassay monitoring has revealed the presence of *C. beticola* isolates resistant to triazoles. Sequencing of the gene *Cyp51,* encoding 14*α*-demethylase, revealed that the presence of several mutations at different loci was associated with reduced sensitivity to triazoles. Since the number of informative loci and the size of *C. beticola* populations are important, a molecular monitoring tool must be able to genotype a large number of loci on a large number of individuals simultaneously. Here, we present the development of a method that takes advantage of high-throughput sequencing to simultaneously detect and quantify *Cyp51* mutations involved in triazole resistance in *C. beticola* populations. After validation on a controlled mixture of strains, this method was used on populations collected in France between 2021 and 2023. The advantages and pitfalls of this method are discussed in the context of fungicide resistance monitoring.

Poster 4

Exploring fungicide resistance in multiple fungal pathogens that infect plants, animals and/or humans

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PHI-base, the Pathogen–Host Interactions Database (www.phi-base.org), is a gold-standard manually curated phenotype database storing molecular information on genes implicated in virulence and/or resistance to chemistry (Urban et al. 2022 PMID:34788826) that aims to help researchers to uncover new fundamental insights and find novel intervention targets for disease control. Our May 2024 release (version 4.17) provides information on 296 pathogens, covering 22,408 interactions, curated from 5,521 peer reviewed articles. We developed an online community curation tool, PHI-Canto (Cuzick et al. 2023 PMID:37401199) (canto.phi-base.org) to permit authors to capture mutant phenotypes from their peer-reviewed articles. PHI-Canto uses controlled vocabularies and ontology terms, including from our newly developed Pathogen-Host Interaction Phenotype Ontology (PHIPO). We selected 80 publications containing information on alterations in fungicide target sites in plant, animal and human fungal pathogens. Altered phenotypes include increased resistance or increased sensitivity to a chemical fungicide treatment. These phenotypes are augmented with information regarding the alteration of the target site, compared to known alterations within designated archetype species of a subset of fungicide targets (obtained from the FRAST database www.frast.com.au and two publications).

Poster 5

Seasonal resistance shifts to pyraclostrobin in populations of *Cercospora beticola* collected from commercial sugar beet fields in the northcentral USA

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Cercospora leaf spot (CLS), caused by *Cercospora beticola* (*Cb*) remains the most important foliar sugar beet disease in the USA and worldwide. Management requires an integrated approach combining resistant varieties, cultural practices and timely field fungicide applications. The fungicides used are protectants from multiple FRAC classes that work best when applied before infection. Resistance has developed to all the fungicides registered in the US for reducing CLS including the QoI fungicide pyraclostrobin despite precautions to reduce resistance. Pyraclostrobin was registered in the US in 2012 with excellent activity against *Cb*, but resistance developed quickly in subsequent years after widespread application. By 2022 more than 90 percent of the spore populations tested (N=1200) were highly resistant to pyraclostrobin at the end of the growing season. Results of a study conducted to compare pyraclostrobin resistance in *Cb* spore populations collected at the beginning and end of three growing seasons showed an average of 77% of the population with high resistance compared to an average of 2.3% at the beginning of the next season. This may indicate a fitness penalty in *C. beticola* isolates resistant to the QoI fungicide pyraclostrobin and may impact fungicide management recommendations.

Poster 6

Twenty-Five Years of Fungicide Resistance Research in *Alternaria solani* in the USA

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Two classes of fungicides affecting mitochondrial respiration, the Quinone outside Inhibitors (QoI, FRAC 11) and succinate dehydrogenase inhibitors (SDHI, FRAC 7), have placed significant pressure on the potato early blight fungus, *Alternaria solani*. The first QoI fungicide was registered in the USA in 1998 and reduced sensitivity to azoxystrobin, pyraclostrobin, and trifloxystrobin developed within 2 years of registration. The F129L mutation was determined to be responsible for the reduced sensitivity with >99% of *A. solani* isolates evaluated containing the mutation. The evolution of reduced sensitivity and resistance in *A. solani* to SDHI fungicides followed a similar trajectory. The first SDHI foliar fungicide, boscalid, was registered in 2005 and resistance detected four years later. At least five mutations have been associated with resistance to the SDHI fungicides. Resistance to SDHI fungicides is also widespread with >90% prevalence in most potato production areas. While reduced sensitivity and resistance to the demethylation inhibitor fungicides (DMI, FRAC 3) and anilino-pyrimidine fungicides (AP, FRAC 9) have been detected in the USA, resistance to these fungicides has not become widespread. Isolates of *A. solani* have been recovered with combined resistances to QoI, SDHI, and AP fungicides with no measurable fitness penalties compared to wildtype isolates.

Poster 7

Multi-Year Monitoring of oxathiapiprolin sensitivity in *Phytophthora infestans* affecting Tomato in Italy

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Phytophthora infestans (Mont.) de Bary is a severe pathogen of tomato (*Solanum lycopersicum* Mill.) causing late blight, a threat to tomato cultivation globally. Effective management of *P. infestans* involves a multi-dimensional integrated approach based on effective chemical fungicides, tolerant or resistant cultivars, cultural practices, decision support systems, and proper postharvest handling. Among fungicides, oxathiapiprolin, a piperidinyl thiazole isoxazoline that inhibits the oxysterol binding protein (OSBP, GROUP 49 FRAC), is notable for its action across all pathogen life stages. Given its classification as a medium-to-high resistance risk fungicide (single site inhibitor) by FRAC, ongoing resistance monitoring is crucial. This study evaluates the sensitivity of *P. infestans* isolates to oxathiapiprolin over multiple years. From 2020 to 2023, 43 tomato leaf samples showing late blight symptoms were collected in the field in various Italian regions. Bioassays were conducted using tomato leaves (cv. Marmande) at oxathiapiprolin (Zorvec™ Enicade® 100OD) concentrations of 0.03 and 1 mg/L. The results revealed reduced sensitivity to oxathiapiprolin in 3 out of 43 samples analyzed during the 2021 and 2022 seasons. The performed multi-year monitoring effectively detected shifts in pathogen sensitivity, providing essential early warnings to help mitigate potential large-scale resistance issues.

Poster 8

Cost-benefit analysis of policy options for combatting anti-fungal/fungicide resistance in the clinic and in agriculture

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The control of fungal human and crop diseases by antifungals = fungicides is undermined by evolved resistance. Cross resistance to the DMIs and DHODH actives used in the clinic and field has been observed for both MOAs. What are the options for limiting the impact of evolved resistance especially as it impacts clinical diseases?

- FRAC-approved strategies - mixtures, alternations, and minimum doses - work but are limited by the small number of MOAs available for use.
- Developing new actives with different MOAs is a slow and expensive process both for agricultural (ca. $300m) and clinical (ca. $1.3bn) use.
- Optimised genetic disease resistance in crops. Genetic disease resistance in crops not only stabilises yields, it reduces fungicide use and prolongs the useful lifespan of fungicide actives. A modest UK investment in breeding and distributing disease resistant crop cultivars would be an effective way to improve not only crop disease levels but also clinical fungal diseases both here and in other countries.
  1. Germplasm repositories, Ca £5m.
  2. Pathogen isolate collections Ca £10m
  3. Publicly funded crop variety trials should include zero and low fungicide use plots. £5m
  4. Subsidised sales of resistant seed would accelerate the adoption of this lines. Ca £5m

Poster 9

Disease severity and conidial production of QoIs-Resistant *Pyricularia oryzae Triticum* isolates on wheat plants treated with Azoxystrobin

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Quinone outside inhibitor (QoI) fungicides have not been effective in controlling the wheat blast disease caused by Pyricularia oryzae Triticum lineage (PoTl) in Brazil. In addition, the G143A mutation in the cytb gene associated with QoIs resistance, higher fitness, and competitive advantages of the QoI-R PoTl remain stable over time. The virulence and conidial production levels of the QoI-R PoTl isolates group under fungicide selection pressure are still unknown. In this study, the disease severity and conidial production of the QoI-R PoTl isolates group were determined on wheat plants treated with azoxystrobin. At a dose of 10 µg mL azoxystrobin, the QoI-R isolate group caused the highest disease severity on wheat leaves (46.1%) on the wheat cultivar Anahuac 75. On the heads, the QoI-R isolate group also caused the highest disease severity value (60.2%). At a dose of 10 µg mL azoxystrobin, the conidia production of the QoI-R isolate group on wheat leaves was significantly higher (1.44 × 106 conidia mL-1) than that of the QoI-S isolate group (1.33 × 104 conidia mL-1). Germination of QoI-R PoTl conidia and production of germ tubes on wheat leaves and heads treated with 10 µg mL-1 of azoxystrobin 6 hours after inoculation (hai) was observed using Scanning Electron Microscopy (SEM). Simultaneously, QoI-S PoTl conidial germination was inhibited on wheat leaves and heads treated with azoxystrobin. Our findings show that QoI-R PoTl isolates were fitter than QoI-S PoTl isolates under fungicide selection pressure in growth chamber conditions. We discuss the ecological implications of the QoI-R PoTl populations adapted in Brazil

Poster 10

Assessing Quinone Outside Inhibitor (QoI) Sensitivity of *Corynespora cassiicola* isolates

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Quinone outside inhibitors (QoIs) represent a critical class of fungicides utilized for managing foliar diseases in soybean crops. Target spot, caused by the pathogen *Corynespora cassiicola*, is a significant foliar disease with considerable economic impact. This study aimed to evaluate the sensitivity of *C. cassiicola* populations to QoIs and to monitor the development of fungicide resistance in soybean fields in Arkansas. Twenty-four isolates of *C. cassiicola* were collected from various fields across Arkansas counties. QoI sensitivity was assessed by determining the effective concentration at which 50% of mycelial growth is inhibited (EC50) using a mycelial growth assay. The assay employed commercial-grade azoxystrobin (22.9% a.i., Syngenta Crop Protection) with concentrations ranging from 0 to 10 mg/L. To inhibit the alternative oxidase pathway, salicylhydroxamic acid (SHAM) was added to all treatment doses. The experiments were conducted in an incubator set at 26°C in the dark. Plates were arranged in a completely randomized design with three replicates per fungicide concentration, with each plate serving as an experimental unit. After five days, the colony diameters were measured, averaged, and analyzed. The average colony diameter data were subjected to analysis using a log-logistic model in R (version 4.1.0) with the Dose-Response Curve (DRC) package. While all isolates exhibited a reduction in mycelial growth, none showed severe inhibition. EC50 values ranged from 0.00009 to 10.0 mg/L (mean = 1.88 mg/L, median = 0.85 mg/L). Molecular analysis using specific primers for the *cytb* gene revealed mutations in the codon G143A in 18 out of 24 isolates, resulting in an amino acid substitution from glycine to alanine, which is associated with QoI resistance. Ongoing monitoring of fungicide resistance is essential for the development of effective resistance management strategies in Arkansas, which will be crucial in addressing future production challenges.

Poster 11

Assessment of sensitivity and fitness of *Alternaria alternata* tangerine pathotype isolates to triazoles, strobilurines and carboxamide

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Alternaria brown spot is a disease caused by the fungus *Alternaria alternata* tangerine pathotype, which causes severe damage in tangerine and hybrid citrus. In Brazil, reports of the pathogen's occurrence have been verified since 2001, and today the pathogen is detected in several fruit-producing states. This study aimed to evaluate the sensitivity of 12 selected isolates from the municipality of Campanha – Minas Gerais State, identified as *A. alternata* through morphological analyses and pathogenicity tests. These isolates were evaluated for their sensitivity to triazoles (DMI – demethylation inhibitors), strobilurins (QoI – quinone outside inhibitors), and carboxamides (SDHI – succinate dehydrogenase inhibitors), as well as potential fitness costs. All isolates showed resistance to QoI fungicides, being resistant to both azoxystrobin and pyraclostrobin with EC_{50} values greater than 1600 $\mu g.mL^{-1}$ and 120 $\mu g.mL^{-1}$, respectively. The isolates were classified as having intermediate resistance to DMI and SDHI fungicides, with average EC_{50} values of 10.58 $\mu g.mL^{-1}$ (difenoconazole), 12.03 $\mu g.mL^{-1}$ (tebuconazole), and 14.26 $\mu g.mL^{-1}$ (boscalid). No reductions in sporulation were observed in the SDHI and DMI-resistant isolates when compared to previously obtained results with sensitive isolates. . However, the mycelial growth of DMI-resistant isolates was reduced, indicating the possibility of fitness costs. SDHI-resistant isolates exhibited hypersensitivity to oxidative stress. The occurrence of triazole, strobilurin, and carboxamide-resistant phenotypes in the sampled areas indicates that anti-resistance strategies should be adopted.

Poster 12

ALS herbicide resistance in tetraploid Stellaria media

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Stellaria media (STEME) is an annual dicot species infesting winter and spring crops. In Europe, 12 cases of herbicide resistance predominately to sulfonylurea herbicides are reported. In 2020, a German-wide survey identified STEME biotypes with resistance to the registered dose of metsulfuron+carfentrazone (5 g ha^{-1} + 20 g ha^{-1}) and/or the herbicide florasulam (5 g ha^{-1}). One biotype, STEME6, showed resistance to metsulfuron+cafentrazone while another biotype, STEME8, showed resistance to both metsulfuron+cafentrazone and florasulam. Molecular analysis showed heterozyogous Pro197Gln and Trp574Leu substitutions for STEME6 and STEME8, respectively. For further studies, we crossed twenty individuals of each biotype to generate the F1 biotypes STEME6-1 and STEME8-1. Subsequent dose-response experiments with STEME6-1 and STEME8-1 confirmed the resistance pattern observed in the parental populations. Two independent experiments with florasulam showed resistance indices of 7.4 and 4.6 and 451.7 and 464.9 for STEME6-1 and STEME8-1, respectively. For metsulfuron+carfentrazone, resistance indices were 82.5 and 262.8 for STEME6-1 and 96.3 and 280 for STEME8-1 in two independent experiments. Our results resemble those by Marshall and colleagues (2010), who found that Trp574Leu but not Pro197Gln substitutions confer resistance to florasulam. Additional studies on the *ALS* gene copies involved in resistance in STEME6-1 and STEME8-1 are under way.

Poster 13

Expanding Virus-mediated reverse genetic techniques to *Lolium* spp. using BSMV vectors

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Herbicide-resistant weeds pose a significant threat to agricultural productivity by reducing the effectiveness of control methods. To combat this, we need to gain a mechanistic understanding of resistance. Genes correlated with herbicide resistance have been identified in key weed species including *Lolium* spp. However, few have been functionally validated and even fewer shown to be necessary or sufficient to confer herbicide resistance. Virus-Induced Gene Silencing (VIGS) transiently reduces gene expression and therefore can be used to functionally validate gene targets in non-model plants. VIGS against *PHYTOENE DESATURASE* (BSMV:asPDS) induces photobleaching in black-grass (Mellando-Sanchez et al., 2020) and wheat (Lee et al., 2015), therefore we hypothesized it could also work in *Lolium*. Inoculation with BSMV:asPDS leads to ~9-13% photobleaching in *Lolium* compared to 80-100% in wheat or black-grass. Therefore, although it is possible to induce photobleaching in *Lolium* via VIGS, these low efficiency rates need to be improved. We are in the process of developing tools aimed at improving the efficiency of VIGS and are working toward using VIGS to test hypothesis about the necessity of specific targets. To test for sufficiency, virus vectors can also transiently drive overexpression *in planta*, and therefore we are testing virus-mediated overexpression (VOX) in *Lolium.*

Poster 14

Early detection of allelic variants endowing acetolactate synthase resistance in four Amaranthus species through an allele specific loop mediated isothermal amplification

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Amaranthus species are troublesome weeds that infest summer crops and are prone to evolve herbicide resistance. In recent years in Italy, in addition to *A. retroflexus* and *A. hybridus*, the spread of *A. tuberculatus* and *A. palmeri* is of concern because these dioecious species have rapid growth and high potential to infest European and Mediterranean countries. All species were confirmed to be cross-resistant to thifensulfuron-methyl and imazamox due to a substitution at codon 574 of the *ALS* gene. The early detection of resistant plants to prevent further resistance spread and facilitate the adoption of the adequate control strategies is fundamental. We developed a rapid diagnostic assay, a multiplex AS-LAMP (Allele Specific-Loop-mediated isothermal AMPlification) to detect the two variants in the *ALS* gene (Leu, TTG and Met, ATG). The results showed that the LAMP primer sets designed were able to recognize the allelic variants in the four Amaranthus species with a single reaction. The assay performance was near 100% for all species and can predict the resistance status within 30 min. This multiplex AS-LAMP is an effective and reliable method that can concretely contribute to manage resistant weed populations and to a more sustainable use of herbicides.

Poster 15

Herbicide resistance investigation in the invasive *Eragrostis plana*

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Eragrostis plana is a perennial grass considered an invasive plant in Southern Brazil. An integrated weed management system is available including glyphosate application. However, reports of failure on glyphosate control and previous dose response studies indicate evolution for herbicide resistance. The objective of this study is to investigate herbicide resistance in a population of *E. plana*, evaluating target-site (TSR) and nontarget-site (NTSR) resistance mechanisms. Leaves from SG_R (R1, R2, R3) and SG_S (S1, S2, S3) population plants were collected 48 and 147 hours after glyphosate spray (720 g a.e ha-1) treatment (HAT). *5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), Aldo-keto reductase (AKR), ABC-C Family MRP10 (MRP10)* and *MRP8 (MRP8)* gene expression analysis were conducted (NTSR). Genomic DNA was extracted for *EPSPS* relative copy number study (TSR). For one (R2) of three SG_R plants EPSPS was upregulated up to 6.3-fold ($P \leq 0.05$) 147 HAT. *AKR, MRP10* and *MRP8* gene expression were also upregulated in 6.4-, 8.3- and 7.6-fold for R2 when compared to SG_S population. All individuals had a similar relative copy-number of the *EPSPS* related to *Tubulin* gene, with one exception (R1), appearing to have higher *EPSPS* copy number. Further studies are being conducting aiming to understand glyphosate resistance evolution in *E. plana*.

Poster 16

First case in Europe of triple resistance to glyphosate, auxins and ALS-inhibitors in a population of Bassia scoparia

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Bassia scoparia is an annual weed common across the world, with herbicide resistance reported mostly in North America. In 2022, seeds were collected from plants in a winter cereal field under no-tillage from Spain, survivors of successive treatments with auxin herbicides and ALS and EPSPS inhibitors. Dose-response assays were performed with these three herbicide modes of action and data collection 28 days after treatment. Values for R and S populations, respectively, were: glyphosate (HRAC 9) doses between 135-2,160 g a.e. ha^{-1} and 33.75-1,080 g a.e. ha^{-1}, GR50 values of 255 and 129 g a.e. ha^{-1}, RF of 2 and LD50 of 389 and 1.240 g a.i. ha^{-1} and RF of 3.2 times; thifensulfuron (HRAC 2) 7.5-480 g a.i. ha^{-1} and 0.24-7.5g a.i. ha^{-1}, GR50 values of 480 and 3.9 a.i. ha^{-1}, RF of 123 and LD50 undetermined due to high survival of the R population; MCPA (HRAC 4) 300-19200 g a.i. ha-1 and 9.4-600 g a.i. ha-1, GR50 values of 389 and 9.1 g a.i. ha-1, RF of 42 and LD50 of 1117 and 19.3 and RF of 58 times. Molecular studies are currently underway to elucidate the resistance mechanism involved.

Poster 17

Herbicidal dsRNA discovery by using virus-induced gene silencing (VIGS) in weeds

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Exploiting plant RNA interference (RNAi) machinery to develop RNA molecules with herbicidal activity sounds a very intriguing possibility. Such dsRNA-based herbicidal formulations would be very impactful in crop protection, not only because it would be a new mode of action, but above all because the so called undruggable targets would become potential targets. Although there are some reports of spray induced gene silencing (SIGS), they mostly refer to plant model species. Therefore, the first step is to find target genes whose silencing cause severe phenotypes in weeds. We are using VIGS (Virus-Induced Gene Silencing) protocols to test the silencing effect of several genes in the model species Nicotiana benthamiana and in two weeds, Solanum nigrum L. and Amaranthus hybridus L. Among the candidate genes, the silencing of PDS (phytoene desaturase) resulted in the typical bleaching phenotype in both N. benthamiana and S. nigrum, whereas A. hybridus resulted very recalcitrant to VIGS, therefore the protocol needs to be fine-tuned. In the next months, other two known herbicide targets, ALS (acetolactate synthase) and PPOX (protoporphyrinogen oxidase), and five other genes (GNOM, CUL1, RH3, SYP71 and MEE5) which encode for proteins that cannot be inhibited by traditional drugs will be evaluated.

Poster 18

Phenotyping of two different 574 allelic variants in *Amaranthus hybridus* ALS inhibitors resistant plants

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PhenAMAre project aimed to give an insight into evolution of resistant biotypes under herbicide selection pressure. The repeated use of ALS inhibitors in soybean has caused the evolution of *A. hybridus* resistant biotypes carrying the very common 574-L (Leu, TTG) and the rare 574-M (Met, ATG) mutations. Did Met evolve from Leu after a single mutation (TTG to ATG) or from the wild-type allele Trp (W) after a double mutation (TGG to ATG)? To characterize this new allelic variant, the homozygous wild-type and mutant progenies were characterized (ALS enzyme activity and genetic background through microsatellite analyses) and then used in different growth experiments. Two early-growth analyses were carried out before a comparative growth experiment using a High Throughput Plant Phenotyping (HTPP) platform (ALSIA - PhenoLab), under ALS selection pressure. The ALS enzyme bioassay indicated that the Met allele endows resistance to higher doses of imazamox compared to the Leu allele. However, both the early-growth and the phenomics experiments, both in presence and absence of ALS selection pressure, indicated that Met causes lower growth compared to Leu. As a follow up experiment seed yields of homozygous progenies and their respective resistance to ALS inhibitors at different growth stages will be examined.

Poster 19

Acetolactate synthase (ALS) resistance, is it a problem in the sugar beet crop?

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Resistance to ALS herbicides has been recorded across a range of grass and broadleaved weeds in England but these have not been identified as a problem in sugar beet crops until 2023. Traditionally, the use of ALS herbicides has been limited in sugar beet compared to other major arable crops with only one ALS active, triflusulfuron-methyl (Debut) having approval for use, this herbicide is almost exclusively used in tank mix with other non-ALS herbicides. SMART beet varieties were first used in England in 2020, these are tolerant to ALS inhibiting herbicides and have approval for foramsulfuron + thiencarbazone-methyl (Conviso® One) to be applied. Label restrictions prevent Conviso® One being applied in tank mix with other ALS or non-ALS herbicides and in 2023 reports of several broadleaved weed populations surviving a Conviso® One application were observed. ADAS Boxworth and British Sugar tested seed from surviving chickweed (*Stellaria media*) and common poppy (*Papaver rhoeas*) following a Conviso® One application, for resistance in a glasshouse pot test. There was a range of control levels seen to Conviso ®One exhibited across the populations tested.

Poster 20

Herbicide-resistant *Amaranthus retroflexus* found in herbicide-tolerant crops

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Amaranthus retroflexus is one of the most economically important weed in the Czech Republic, especially in sunflower, sugarbeet, potato and vegetable. In recent years in several farms, we have registered the occurrence of *Amaranthus retroflexus*, whose plants survived herbicide treatment in herbicide-tolerant crops (HT technology), which are based on tolerance to ALS inhibitors. Seeds from four populations of *Amaranthus retroflexus* were collected from HT sunflower. Efficacy of tribenuron, foramsulfuron, thiencarbazon, triflusulfuron and nicosulfuron was tested in pot experiment. The two populations (R1 and R2) that were confirmed to be resistant to all tested active ingredients were subjected to a dose response test with six increasing doses of foramsulfuron + thiencarbazone. Furthermore, the possibility of increased metabolism of herbicide mediated by CyP450 and GST was studied using malathion and NBD-Cl at a dose of 1000 g ha^{-1} and 270 g ha^{-} respectively. The dose-response experiments showed high resistance of both biotypes to foramsulfuron + thiencarbazone, with resistance factors (RF) of 351.67 and 12.5 respectively. Upon pre-treatment with malathion, RF decreased 2 times in R1 biotype (RF-172), indicating possible cytochrome P450 involvement. Pre-treatment with NBD-Cl RF led to increased levels of RF in both biotypes, suggesting no possible involvement of the GSTs.

Poster 21

Lolium and *Festulolium*: a growing concern as herbicide-resistant weeds

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Interspecific hybridization serves as a widely utilized technique in forage grass breeding. *Festulolium* hybrids, known for their high yield, digestibility, and palatability, exhibit rapid and vigorous spring growth. However, there has been an observed phenomenon of *Festulolium* escaping cultivation and transitioning into weed populations. Among these weeds, the genus *Lolium* is particularly affected by herbicide resistance on a global scale. While fescue species carry resistance genes to herbicides, there exists a potential risk of the spread of naturally occurring tolerance of *Festuca* species to ACCase inhibitors through hybridization. This risk is exacerbated by the increasing utilization of *Festulolium* hybrid varieties as catch crops in the Czech Republic. Experiments conducted in pots have tested the susceptibility of various *Festulolium* varieties and field samples of *Lolium multiflorum* to herbicides such as cycloxydim, pinoxaden, and propaquizafop. Field samples of *L. multiflorum* displayed resistance to all three herbicides. The involvement of Cyp450 monooxygenases was confirmed in pinoxaden and cycloxydim resistance, while glutathion-S-transferases was found to play a role in cycloxydim resistance exclusively. *Festulolium* has exhibited higher tolerance or resistance to all tested compounds compared to *Lolium* species. There is a notable risk that herbicide-resistant weeds may spread within agroecosystems through the process of hybridization.

Poster 22

Controlling Non-Target Site Resistant *Poa Annua* with Novel Modes of Action

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Several herbicide-resistant *Poa annua* biotypes were challenged with herbicides from Group 30 and Group 28 in dose-response experiments at the University of Tennessee. Four biotypes were included that are resistant to herbicides from Groups 2, 3, 5, and 29 via both target- and non-target site mechanisms. An herbicide-susceptible biotype (S1) with no history of exposure to the same herbicide groups was included for comparison. All *P. annua* biotypes were challenged with 0, 0.125, 0.25, 0.50, 1, 2, 4, 8, and 16x rates of cinmethylin, methiozolin, and tetflupyrolimet applied preemergence. Aboveground biomass data were expressed as a percentage of the non-treated and subjected to non-linear regression to determine EC_{50} values for each herbicide. Cinmethylin, methiozolin, and tetflupyrolimet effectively controlled resistant *P. annua*. EC_{50} values ranged from 25 to 93 g ha^{-1} for cinmethylin, 34 to 137 g ha^{-1} for methiozolin, and 20 to 52 g ha^{-1} for tetflupyrolimet. While these herbicides exhibit preemergence efficacy, EC_{50} values for each herbicide applied to susceptible *P. annua* were $\leq$ 0.02 g ha^{-1}. Reduced sensitivity of the resistant biotypes suggests that sole reliance on cinmethylin, methiozolin, or tetflupyrolimet could eventually select for accessions with the ability to survive treatment at field rate in the future.

Poster 23

Syngenta glasshouse-based testing platform for developing herbicides that can control evolved resistance in weeds

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Globally, resistance is on the rise for many persistent weed populations, resulting in significant reductions of crop yields. Syngenta has established a pipeline for identifying, monitoring, and screening novel resistance mechanisms. This includes purifying seed populations from parental lines sourced from a diverse range of cropping systems and selection pressures, screening these against a portfolio of novel herbicides, and using the data to inform chemical design. This is achieved through recent development of a high-throughput glasshouse screening platform which utilises purified seed stocks of both target site and non-target site resistant lines. With a focus on data quality, this approach allows a comprehensive dose response over many herbicide rates, in addition to double the replication while using only a third of the glasshouse space compared to standard regular glasshouse screens. Supplying relevant and timely seed lines for a specifically designed high-throughput resistance screening platform allows our research to better understand and address the global issue of resistance increasingly faced by growers.

Poster 24

The potential of using natural synergists in integrated pest management of Cabbage stem flea beetle (*Psylliodes chrysocephala*)

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In the UK the area used for growing oilseed rape has fallen dramatically in recent years due to infestations of Cabbage Stem Flea Beetle (CSFB, *Psylliodes chrysocephala*). This is largely the result of the inability of farmers to control CSFB populations due to the banning of effective insecticides (neonicotinoid seed treatments) and to the development of resistance to pyrethroids (the only other permitted insecticide registered for control). Effective pest management options are urgently required. Synergists are used extensively in the household and vector control markets to enhance the efficacy of insecticides, especially when problems of resistance are present. In this study, a novel natural synergist was tested to evaluate the synergistic effect on the pyrethroid lambda-cyhalothrin, and its effects on both CSFB and the parasitoid of its adult stage (*Mictoctonus brassicae*). This new synergist was shown to inhibit the metabolic enzymes responsible for conferring insensitivity to pyrethroids in CSFB, and thus increased their sensitivity to lambda-cyhalothrin by 280% and 375% in glass vial assays and plant feeding assays, respectively. However, this effect is also seen in the parasitoids, increasing their sensitivity to pyrethroids by 300%. The potential of using synergists for CSFB control as part of an integrated pest management strategy is discussed.

Poster 25

An experimental-theoretical approach to understand and predict pesticide resistance evolution in *C. elegans*

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Widespread application of agricultural pesticides has led to the emergence of resistance, with resulting failures in pest management leading to crop loss valued in the billion, thus posing grave challenges to global food security. To mitigate the effects of evolved resistance, it is important to fully understand the dynamics of resistance evolution. However, our current knowledge is primarily limited to insights derived from mathematical and computational models. Experimental insights into resistance evolution are sparse, and existing studies focus on specific pest and pesticide biology. The field will benefit significantly from an evolutionarily transparent experimental model which can iterate with computational predictions. The nematode *Caenorhabditis elegans* is ideal for this due to its advantageous life-history traits. We built a transparent and tractable experimental model of pesticide resistance evolution in *C. elegans*. Our model captured the expected outcomes using an experimental evolution approach in which resistant and sensitive genotypes were competed. We also show that a companion computational model was able to predict *in vitro* evolution dynamics when parameterised with experimental data. In sum, we have made the first steps towards constructing an experimental-theoretical paradigm for understanding and predicting pesticide resistance evolution which we anticipate will have extensive applicability.

Poster 26

How a science-driven marketing-forward approach is addressing an evolving crisis

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In 2015, results of market research revealed that North American soybean growers were largely unaware that overuse of the same source of genetic resistance (PI88788) to the soybean cyst nematode (SCN), *Heterodera glycines*, was leading to an economic crisis. Without intervention, losses to this billion-dollar threat would continue to increase. In 2018, a public-private partnership (PPP) of soybean grower organizations, universities and agro-seed - chemical companies was launched, with the mission to increase grower awareness and active management of SCN. Using a science-driven marketing-forward approach, SCN Coalition messages generated millions of video views and hundreds of millions of potential impressions through the agricultural media. A repeat of market research in 2020 found that grower recall of SCN Coalition messaging ranged from 65%-76% and documented statistical increases in growers use of genetic resistance (6%), rotation of genetic resistance (10%), crop rotation (6%) and use of seed treatment nematicides (18%). The estimated economic savings exceeded \$100M by the end 2020, and will be measured again in 2024. The SCN Coalition has been honored with prestigious awards in marketing and public relations for altering the trajectory of this looming crisis, and may serve as a model for addressing pest evolution and pesticide resistance.

Poster 27

Teasing apart the contributions of the different mechanisms involved in *Myzus persicae* resistance to neonicotinoid

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Insecticide resistance can lead to the repeated application of treatments that are both ineffective in pest control and harmful for the environment. In order to establish sound strategies to slow down or limit these resistance phenomena, precise knowledge of the underlying mechanisms, their relative contribution and their potential interaction is essential. *Myzus persicae* is a major pest aphid, capable of infesting a wide range of crops, with significant economic impact. *M. persicae* is known to have evolved resistances to insecticides of different families, including the abundantly used neonicotinoids. *M. persicae* resistance to neonicotinoids has previously been described as being due to two main mechanisms: a P450 overexpression metabolic resistance and a target-site mutation, R81T, but their respective contribution to resistant phenotypes remains unclear. We combined extensive bioassays with synergist on numerous clones, with gene copy number and expression quantification of two key P450 enzymes (*CYP6CY3* and *CYP6CY4*) to explain the observed phenotypes and assess the relative contribution of metabolic and target-site mechanisms to neonicotinoid resistance in *M. persicae*.

Poster 28

Mosquito ABC Transporter Modulation and Strategies to Improve Insecticide Delivery

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There is a need to develop new tools to fight mosquito-borne pathogen transmission in the absence of a globally available vaccine and wake of insecticide resistance leading to product failures for reducing mosquito population densities. The mosquito central nervous system is a proven target site for high efficacy insecticides. However, widespread resistance and inefficient target-site delivery limits current insecticides to reduce mosquito population densities and prevent disease transmission. Thus, insecticide resistance and target-site delivery are serious public health challenges that warrant the development and delivery of improved chemical strategies. ATP-binding cassette (ABC) transporters are integral membrane proteins at the blood-brain barrier to traffic out insecticides and reduce their intracellular delivery to target sites. Data will provide evidence of a blood-brain barrier obstacle for the intracellular delivery of insecticides to the central nervous system. A series of ABC transporter-targeting chemistries will be shown to modulate the activity of insecticide modes of action. It will also be reported the spatiotemporal dynamics of ABC transporters in response to insecticide exposures. Lastly, an innovative attractive targeted bait technology will be discussed as an insecticide delivery strategy to enhance the effective area of activity to reduce mosquito population densities for the prevention of malaria transmission.

Poster 29

Unravelling the Genetic Architecture of Flubendiamide Resistance in Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae)

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Insecticide resistance significantly impacts food production, environmental sustainability, and human health. Helicoverpa armigera (Lepidoptera: Noctuidae), a major crop pest, rapidly develops insecticide resistance. A previous study showed that the flubendiamide resistance in this H. armigera strain is not associated with mutations in the ryanodine channel gene or an increase in enzymatic detoxification. Thus, we explored the genetic underpinnings of H. armigera resistance to flubendiamide using high-resolution Genome-Wide Association Studies (GWAS) and Quantitative Trait Loci (QTL) mapping. GWAS identified six loci, one on chromosome 1, three on chromosome 2 and two on chromosome 13, that correlate with reduced susceptibility to flubendiamide. Concurrent QTL mapping pinpointed a major effect QTL on chromosome 2 associated with resistance traits. The identified locus on chromosome 2 by GWAS and QTL mapping is promising to develop molecular markers for resistance monitoring. The identified candidate genes may be associated with maintaining calcium homeostasis in the cell and insecticide detoxification by the ATP-binding cassette (ABC) family genes. Our findings suggest an oligogenic architecture and a different mechanism of H. armigera resistance to flubendiamide in Brazil. Furthermore, these results contribute to developing molecular markers to effective insecticide resistance management strategies.

Poster 30

The overexpression of cytochrome P450 genes is associated with flonicamid resistance in the green peach aphid, *Myzus persicae*

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Flonicamid, a selective aphicide with excellent activity against the aphid pest, *Myzus persicae* that is not compromised by pre-existing resistance to other insecticides with different modes of action. However, some signs indicate that the efficacy of flonicamid against *M. persicae* is decreasing due to the emergence of resistance. Therefore, investigation of the molecular mechanisms underpinning resistance is urgently required. Insecticide bioassays using the P450 inhibitor piperonyl significantly synergized the toxicity of flonicamid in two resistant clones of aphids compared to a susceptible clone, suggesting that P450s play a role in resistance to this compound. Transcriptomic comparative analysis between two resistant clones (SYN4 and SYN9) and four susceptible clones (4255A, US1L, 1X, and 23) identified 336 genes that were commonly upregulated in resistant clones, including eight cytochrome P450 genes. Subsequently, qPCR validation revealed that three of the P450 genes, *CYP6CY3*, *CYP6CY4*, and *CYP6CY36*, were significantly overexpressed in both the SYN4 and SYN9 clones compared with the 4255A clone. Further functional analyses showed that only transgenic *Drosophila melanogaster* expressing *CYP6CY3* and *CYP6CY4* exhibited significant resistance to flonicamid. Our findings provide a evidence that the overexpression of *CYP6CY3* and *CYP6CY4* contributes to flonicamid resistance in *M. persicae*.

Poster 31

EPPO Database on Resistance Cases to Plant Protection Products

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The EPPO Database on Resistance Cases is a collection of more than 800 documented resistance cases of diseases/pests/weeds to Plant Protection Products (PPPs) based only on occurrence detected in the field. It provides information to authorities responsible for registration of PPPs and other stakeholders such as the agrochemical companies across the EPPO region (52 countries) which apply for registration of PPPs, researchers, scientists, extension officers and users of EPPO Standard PP 1/213 *Resistance risk analysis*. It also facilitates a common understanding of evolution of resistance to PPPs across the EPPO region, including the early identification of resistance trends, and helps to develop resistance management strategies that consider both the national and regional resistance contexts. The added value of the Database, compared to other available databases, is the inclusion of information about early detection of resistance cases, as well as resistance cases that occur locally, that are based on data even if this data is limited. Since it was launched in 2021, the Database is updated annually with new cases. The Database already lists cases from 18 countries and is expected to become an important reference Database for resistance cases within the EPPO region over the coming years.