727F **Fungal G-protein coupled receptors promote Fusarium Head Blight disease on wheat**. **T Dilks1 , K Halsey1 , R de Vos1 , K Hammond-Kosack1** , N.A. Brown2 **1) Biointeractions and Crop Protection, Rothamsted** Research, Hertfordshire, UK; 2) Biology and Biochemistry, University of Bath, Bath, UK. Fusarium Head Blight (FHB) is the number one floral disease of cereals and poses a serious health hazard by contaminating grain with the harmful mycotoxin deoxynivalenol (DON). Fungi adapt to fluctuations in their environment, coordinating development and metabolism accordingly. G-protein coupled receptors (GPCRs) communicate changes in the environment to intracellular G-proteins that direct the appropriate biological response, suggesting that fungal GPCRmediated sensing may be key to virulence. Here we describe the expansion of non-classical GPCRs in the FHB causing pathogen, Fusarium graminearum, and show that class X receptors are highly expressed during wheat infection. We identify class X receptors that are required for FHB disease on wheat, and show that the absence of a GPCR can cause an enhanced host response that restricts the progression of infection through the apoplast, while specific receptor subdomains are required for virulence. These non-classical receptors physically interact with intracellular G-proteins and are therefore bona fide GPCRs. A class X receptor is shown to regulate transcriptionally the coordination of isoprenoid metabolism and downstream virulence traits during infection. This amounts to enhanced wheat defensive responses to infection, including chitinase and plant cell wall biosynthesis. Our results show that GPCR-mediated sensing is important to FHB disease establishment.

763F **Analysis of small RNAs in the Zymoseptoria tritici – wheat interaction**. **G.J. Kettles1,4, B.J. Hofinger1 ,** P. Hu2 **, C. Bayon1 , J.J. Rudd1 ,** D. Balmer3 **,** M. Courbot3 **, K.E. Hammond-Kosack1 ,** G. Scalliet3 **, K. Kanyuka1 1) Biointeractions & Crop Protection, Rothamsted** Research, Harpenden, United Kingdom; 2) Syngenta Biotechnology Inc., Research Triangle Park, North Carolina, USA; 3) Syngenta Crop Protection AG, Stein, Switzerland; 4) School of Biosciences, University of Birmingham, Birmingham, United Kingdom. Cross-kingdom small RNA (sRNA) silencing has recently emerged as a mechanism facilitating fungal colonisation and disease development in plants. Here we characterise RNAi pathways in Zymoseptoria tritici, a major fungal pathogen of wheat, and assess their contribution to pathogenesis. Computational analysis of fungal sRNA and wheat mRNA sequencing datasets was used to define the global sRNA populations in Z. tritici and predict putative mRNA targets in wheat. In total, 389 in planta-induced sRNA loci were identified in Z. tritici. sRNAs generated from some of these loci were predicted to target wheat mRNAs, including some that have previously been implicated in defence against pathogens. However, biochemical approaches were unable to successfully validate targeting of selected wheat mRNAs by fungal sRNAs. Z. tritici gene deletion strains deficient for key RNAi components were generated and virulence bioassays suggested that these are dispensable for full infection of wheat. Nonetheless, our results do point to the existence of non-canonical Dicer-independent pathway(s) for sRNA biogenesis in Z. tritici. dsRNA applied in vitro or generated from an RNA virus vector in planta was ineffective at triggering gene silencing or reducing growth of Z. tritici. We conclude that neither in vitro nor in planta RNAi approaches are likely to be useful for gene function analyses or as a viable control measure for this pathogen.

785W **Investigating the role of autophagy in supporting early symptomless colonisation of Zymoseptoria tritici on wheat.** H.T. Child1 **, J.J. Rudd2** , M.J. Deeks1 , S. Bates1 , K. Haynes1 1) Biosciences, University of Exeter, Exeter, Devon, UK; **2) Department of BioInteractions and Crop Protection, Rothamsted** Research, Harpenden, Hertfordshire, UK. The fungal wheat pathogen Zymoseptoria tritici is responsible for major crop losses through causing the disease Septoria leaf blotch (STB). Mitigating this disease is of major economic importance, with an estimated annual fungicide input worth £1 billion targeted to controlling STB in Europe. The infection cycle of Z. tritici displays two distinct phases, beginning with an extended symptomless phase as the spore germinates and extends hyphae across the leaf surface and into stomata. Following a long slow intercellular growth phase (> 7 days), the fungus then induces host cell death and tissue collapse in the leaf. Recent evidence from transcriptome analysis suggests that the fungus uses little host-derived nutrition during the early stages of infection, instead relying on macromolecules within the fungal spore. Our research aims to elucidate how Z. triticiremobilises stored nutrients to support growth during colonisation of the wheat leaf. We are currently investigating whether Z. tritici uses the self-degradative process of autophagy to recycle cellular resources for germination and growth during the initial symptomless phase. The key autophagy genes ATG1,

encoding a protein kinase involved in autophagy initiation, and ATG8, encoding a ubiquitin-like protein required for autophagosome formation, have been characterised by gene deletion to assess the importance of autophagy to Z. tritici cellular differentiation and virulence. We have also generated Z. tritici strains expressing Atg8 tagged with GFP to visualise the spatial and temporal occurrence of autophagy during growth and differentiation. We present the current status of this project which will provide fundamental knowledge of the molecular mechanisms of Z. tritici infection, as well as identifying potential new targets for control of this devastating disease.

791W **Using Virulence Mutants to Identify Avr Genes in the wheat stem rust fungus, Puccinia graminis f. sp. tritici**. Peter Dodds1 , Narayana Upadhyaya1 , Diana Oriz1 , Feng Li2 , Jiapeng Chen3 , Marisa Miller2 , Ming Luo1 , Rohit Mago1 , Jeff Ellis1 , Jana Sperschneider1 , Hoa Nguyen-Phuc2 , **Clement Bouton4** , Brian Steffenson1 , Cory Hirsch1 , Kevin Silverstein5 , Robert Park3 , Kostya Kanyuka5 , Melania Figueroa2 1) Agirculture and Food, CSIRO, Canberra, ACT, AU; 2) Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA; 3) University of Sydney, Plant Breeding Institute, Narellan, NSW, Australia; **4) Rothamsted Research, Biointeractions and Crop Protection**, Harpenden, United Kingdom ; 5) Minnesota Supercomputing Institute, The University of Minnesota, Minneapolis, MN, USA. The wheat stem rust fungus Puccinia graminis f. sp. tritici (Pgt) is one of the most destructive pathogens of wheat. Resistance of host lines is often governed by recognition of fungal effector proteins (avirulence/virulence proteins) by plant resistance proteins (R proteins). We have taken a mutational genomics approach to identify Avr genes in Pgt. We isolated spontaneous mutants with virulence for Sr50, Sr5, Sr27, Sr21 or Sr45 by selection on resistant host lines. Sequence analysis of the Sr50 virulent mutant revealed that virulence resulted from the exchange of a whole chromosome between the two haploid nuclei of this dikaryotic organism, resulting in loss of the avirulence allele. This confirms the important role of somatic exchange events in virulence evolution in Pgt. The AvrSr50 gene was identified from the 25 candidate effector genes from this chromosome by transient co-expression with the cloned Sr50 gene in N. benthamiana. AvrSr50 recognition was confirmed in wheat by viral expression. Recognition of the AvrSr50 protein by the host Sr50 immune receptor is based on direct interaction and we have identified critical amino acid polymorphisms contributing to the escape from recognition in virulent isolates. Identification of AvrSr50 has enabled development of tools for testing effector function in wheat including viral overexpression and wheat protoplast transient expression assays. Spontaneous mutants for several other Avr loci have also been sequenced and a new Pacbio-based genome assembly for the Australian parental Pgt isolate has facilitated the delineation of these loci. Three mutants with virulence for Sr27 contain overlapping deletions and a single candidate gene for AvrSr27 has been identified. Likewise, AvrSr5 mutants contain large deletions spanning several candidate effector genes.