

Abstracts

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Oral Presentation Abstracts

[O1] The Changing Landscape of Clubroot in Canola: Virulence Shifts and Management Challenges

Stephen Strelkov and Sheau-Fang Hwang

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5

Correspondence to: strelkov@ualberta.ca

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae*, is a significant soilborne disease affecting the Brassicaceae family. Although clubroot has been present on cruciferous vegetables in some regions of Canada for over a century, it was first detected on canola (*Brassica napus*) in the Canadian Prairies in 2003, when 12 *P. brassicae*-infested fields were found in central Alberta. Over the past two decades, the disease has continued to spread, with more than 4,100 confirmed field infestations across most of Alberta by 2024, as well as detections in Saskatchewan, Manitoba, and North Dakota, USA. Managing clubroot is challenging, as *P. brassicae* produces large numbers of long-lived resting spores that persist in the soil for many years. Control strategies for clubroot in canola rely heavily on resistant cultivars; however, intensive cultivation of resistant varieties has led to the emergence of resistance-breaking pathotypes capable of causing severe disease on previously resistant canola. These changes in pathogen virulence have resulted in shifts in the predominant pathotypes in western Canada. Prior to the deployment of clubroot-resistant cultivars in 2009, pathotype 3H (as defined by the Canadian Clubroot Differential set) was dominant but has since been largely supplanted by resistance-breaking pathotypes such as 3A and 3D. Ongoing efforts aim to enhance our understanding of clubroot biology and management, including refining host differential sets and developing metabarcoding strategies for pathotype identification. Ultimately, an integrated clubroot management approach, incorporating multiple strategies, would be more sustainable in mitigating the impact of the disease.

[O2] Pangenomic and virulence analyses of the barley leaf rust pathogen, *Puccinia hordei*

Brian J. Steffenson¹, Rebecca Spanner¹, Eric S. Nazareno¹, Eva Henningsen², Jibril Lubega³, Kostya Kanyuka³, Matthew Moscou⁴, Matthew N. Rouse⁵, Jana Sperschneider⁶, Peter Dodds⁶, and Melania Figueroa⁶

¹Department of Plant Pathology, University of Minnesota, St. Paul, MN; ²Research School of Biology, The Australian National University, Canberra, ACT, Australia; ³National Institute of Agricultural Botany (NIAB), Cambridge, Cambridgeshire, United Kingdom; ⁴USDA-ARS Cereal Disease Laboratory, St. Paul, MN; ⁵USDA-ARS Sugarcane Field Station, Canal Point, FL;

⁶Commonwealth Scientific and Research Organization, Canberra, ACT, Australia

Correspondence to: Brian J. Steffenson at bsteffen@umn.edu

Barley leaf rust, caused by *Puccinia hordei* (*Ph*), is a widespread disease throughout world and can cause yield losses as high as 60% in highly susceptible cultivars. To gain a greater understanding of the genomics and virulence dynamics of *Ph*, a worldwide collection of over 400 isolates was assembled. Ten isolates from this panel were selected for PacBio HiFi and Hi-C sequencing based on their geographic origin, previous genotyping data, and virulence patterns on the Bowman leaf rust differential lines carrying the resistance genes of *Rph1* to *Rph15*. Six of the sequenced isolates were from the U.S. (from CA, MN, VA, TX, and WA), and one each was from China, Germany, Israel, and the Netherlands. A *de novo* reference genome for the U.S. isolate 17MN32B was assembled based on synteny with the haplotype-phased *P. graminis* f. sp. *tritici* isolate Pgt21-0, which then guided the scaffolding of the remaining isolates. Half of the assemblies achieved telomere-to-telomere completeness with 9/10 assemblies retaining over 34/36 telomeres. Nuclear genome sizes ranged from ~140–147 Mb apart from the Israeli isolate ISR90-3 which had a markedly larger genome size of ~163 Mb. Out of the 20 assemblies, 13 unique nuclear haplotypes were identified. Clonality was evident in the U.S. isolates, reflecting the limited presence of the alternate host (*Ornithogalum* species) and its minimal role in the sexual reproduction of the pathogen. Haplotype containment analyses revealed multiple somatic hybridization events that have shaped global *Ph* lineages. This contention is based on the detection of a single haplotype (hapB) that was shared among four U.S. isolates and also an Australian isolate which may indicate an incursion. *Ph* genomes exhibited a higher repeat content (~70%) than other related haplotype-phased cereal rust fungi, with expansions in LTR retroelements and DNA transposons. Additionally, putative reciprocal translocations were observed in four isolates. Gene annotations are underway using germinated spore and infection RNAseq data from an isolate with near-universal avirulence on the Bowman leaf rust differential lines. These annotated phased genomes provide a crucial foundation for nuclear surveillance and Avr gene discovery in this pathosystem. With respect to latter objective, Illumina sequencing will be performed on 60 diverse *Ph* isolates from our collection as well as 100 other isolates derived from a sexually-reproducing population collected from *Ornithogalum* in Israel. The sequencing data on these isolates together with their virulence phenotypes on the Bowman differential lines and an extended set of other barley lines possessing the resistance genes *Rph16* to *Rph24* will facilitate the discovery of effector gene in *Ph*.

[O3] Development and evaluation of screening methods under a controlled environment for pasmo resistance in flax

Keiko Nabetani¹, Ashley Smith¹, Bunyamin Tar'an¹, Randy Kutcher¹

¹Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8

Pasmo is one of the most prevalent and damaging diseases of flax in western Canada. Effective host resistance is constantly searched for; the field disease nursery is the main method used to screen for resistance. However, high variability in assessment has been an issue, and a better screening method is needed. In this study, multiple trials were conducted in growth chambers to determine the optimal spore concentrations, flax growth stage, incubation temperature and incubation duration for pasmo resistance screening under a controlled environment. In trial #1, four spore concentrations (2.5x10⁴, 2.5x10⁵, 2.5x10⁶, and 2.5x10⁷ spores/mL) were tested by

inoculating two flax genotypes, 'CDC Bethune' (moderately susceptible) and L.00-207 (CN 101299, moderately resistant) at the seedling and flowering stages. In trial #2, three incubation temperatures (18, 22 and 26°C) and four post-inoculation incubation durations under high humidity (0, 24, 48 and 72 hours) were tested by inoculating two flax genotypes at the seedling stage. At the flowering stage inoculation, two highest spore concentrations resulted in higher stem lesion severity on 'CDC Bethune' than L.00-207. At the seedling stage inoculation, 'CDC Bethune' had higher stem severity than L.00-207 when stem lesion severity was averaged over all inoculum concentrations. There was no difference between the two flax genotypes when the lower incubation temperature and longer incubation time were used. These results indicate that indoor screening with high inoculum concentration could identify host resistance against pasmo; however, more trials are needed to refine the protocols for consistent results.

[O4] Enhancing Canola Resistance to Verticillium Stripe: qPCR-Based Phenotyping and GWAS Reveal Genetic Markers for Breeding Applications

Mohamed Samir Youssef¹, W. G. Dilantha Fernando¹, Robert Duncan¹, Sally Vail², Isobel A. P. Parkin², Harmeet Singh Chawla^{1*}

¹Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, Canada, R3T2N2; ²Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

*Correspondence to: harmeet.chawla@umanitoba.ca

Verticillium longisporum (VL), a soil-borne vascular fungal pathogen, poses a significant threat to cruciferous crops, including canola (oilseed rape), and can cause up to 80% yield loss under severe infestations. VL enters plants through the roots and colonizes the vascular system, leading to Verticillium stripe (VS) disease, which was first detected in Canada in 2014. This disease has emerged as a major concern for Canadian canola production, with its severity expected to increase due to rising temperatures driven by climate change and increasing inoculum loads in VL-infested fields. Developing canola cultivars with resistance to VS is therefore critical to safeguarding yield potential for Western Canadian growers. A unique characteristic of this pathogen is its systemic, non-homogenous, and delayed colonization of the plant xylem, leading to an extended symptomless latency period. Consequently, the severity of infection in the field is challenging to assess, as symptoms become apparent only at crop maturity, and are often confused with natural senescence. Traditional methods, such as visual scoring of microsclerotia on harvested stubble, are unreliable indicators of genotypic resistance, as they are strongly influenced by plant maturity stages. To overcome these limitations, we aim to improve the phenotyping process by developing a quantitative PCR (qPCR) method capable of accurately distinguishing levels of quantitative resistance to *V. longisporum* in canola under field conditions. In this study, we are screening a diverse panel of 216 *Brassica napus* genotypes representing a wide genetic base sourced from various seed companies and research institutions. These genotypes were evaluated in field trials, and stem tissue samples were collected to quantify VL presence using qPCR. Genome-wide association studies (GWAS) revealed three significant marker-trait associations (MTAs) related to VS resistance, offering insight into the genetic basis of

resistance. In ongoing and future trials, we will validate these findings and further elucidate resistance mechanisms. Once key polymorphisms, such as SNPs and Indels within the resistance QTLs, are identified, we will develop KASP or PCR-based markers to facilitate the introgression of VS resistance alleles into elite Western Canadian breeding lines and cultivars. This work aims to deliver robust molecular tools for breeding VS-resistant canola, thereby enhancing yield stability and production sustainability in Western Canada.

[O5] Accelerated Clubroot Resistance Breeding in *Brassica napus* using Optimized Double Haploid Technology and Genetic Mapping

Xinlong Dong, Rudolph Fredua-Agyeman, Sheau-Fang Hwang and Stephen E. Strelkov

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5

Correspondence to: strelkov@ualberta.ca

Clubroot disease, caused by *Plasmodiophora brassicae*, poses a severe threat to Canadian canola (*Brassica napus*) production, with pathotypes 3H and 3A among the most common. Given the rapid shifts in the virulence of the clubroot pathogen, there is an urgent need to accelerate the development of cultivars with novel resistance sources. This study integrates molecular breeding with genetic mapping to expedite resistance breeding. An optimized doubled haploid (DH) method using microspore culture was developed, achieving a high plantlet regeneration efficiency of 79%, significantly surpassing previously reported rates of 5%–30%. This method was applied to 29 F1 hybrids from 11 diverse crosses between five male and three female *B. napus* plants, generating a total of 5,728 DH plantlets. A subset of 189 DH lines from a single cross population, along with their parental lines, were tested for resistance to *P. brassicae* pathotypes 3A and 3H. Chi-square analysis confirmed a 1R:1S segregation ratio for both pathotypes, indicating the presence of a major dominant resistance gene. Previous screening of the F2 generation from the same cross, which showed a 3R:1S segregation ratio, further supported the presence of this gene. Future work will focus on constructing a high-density linkage map and performing QTL analysis to localize this major resistance locus. By combining an efficient DH method with genetic mapping, this research aims to accelerate the development of clubroot-resistant cultivars by rapidly generating pure lines, enabling precise phenotyping, and facilitating marker-assisted selection.

[O6] Exploiting Susceptibility Genes in Canola to Improve Blackleg Disease

Anuradha De Silva¹, Shuanglong Huang¹, Raju Datla², Gary Peng³, and Dilantha Fernando¹

¹Department of Plant Science, Faculty of Agriculture and Food Sciences, University of Manitoba, Winnipeg, MB, Canada, R3T2N2; ²Global Institute for Food Security, Saskatoon, Saskatchewan, SK, MB, Canada, S7N4L8; ³Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, SK, MB, Canada, S7N 0X2

Correspondence to: dilantha.fernando@umanitoba.ca

Blackleg is the most widely occurring disease on canola in western Canada, *Leptosphaeria maculans* is the major causal organism and it affects yield losses and trade disputes in export.

While integrated blackleg management strategies contain various approaches, genetic resistance remains fundamental, primarily relying on specific resistance (R) genes and quantitative resistance (QR). However, limited availability of effective R genes is a major concern, as virulent pathogen races capable of overcoming nearly every known R gene already exist within the region. Therefore, the durability of utilizing R-gene resistance is uncertain. Although, QR contributes to blackleg resistance in many Canadian cultivars, its complex presents considerable challenges for targeted improvement through breeding. Alternatively, resistance to crop diseases can be achieved through functional disruption of susceptible (S) genes. These S-genes play a crucial role in establishing compatibility between the host and pathogen during their interaction, thereby facilitating the infection. This study identifies S-genes associated with blackleg pathogen virulence and disease severity. A two-step strategy is used by screening a unique canola TILLING (Targeting Induced Local Lesions in Genomes) population available at the Global Institute for Food Security to identify prospective S gene candidates. Based on the young plant (cotyledon) resistance screening, three potential mutagenized lines were determined to confer resistance against *L. maculans*. These mutagenized lines will be further studied to identify and map the candidate S-genes.

[O7] Metabarcoding of Aeromicrobiomes and Insect Trap Preservative Screens for Phytopathogenic Spores in Agricultural Fields: Building a Predictive Tool

Émilie D. Tremblay¹, Étienne Lord², Benoit Goulet¹, Stéphane Samson², Jean-Philippe Parent², Sarah Hambleton¹ and Julie Carey¹

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, Canada, K1A 0C6; ²Saint-Jean-sur-Richelieu Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Blvd, Saint-Jean-sur-Richelieu, QC, Canada, J3B 3E6

Human, animal, and plant health depend on ecosystems that are regulated by climate, environmental factors, and the aerobiome. While some microbes are essential for plant health, invasive fungal plant pathogens pose significant risks to Canadian crop production (quality and yields). The aerial spores of these pathogens can spread and infect host plants, a phenomenon aggravated with ever increasing and expanding imports that can introduce alien fungal spores. This project collected five years' worth of environmental samples (N=1635, 4-8 fields/farm, across four AAFC Farms in QC and ON) for early detection of invasive fungal pathogens to establish baseline data for a predictive tool. While passive spore traps were used to assess the aeromicrobiome, aphids suction traps' preservative fluids were used to assess the passive suction or transport of fungal spores by insects to host plants. Microbial communities obtained through these methods varied significantly across the multiple farmlands studied. Metabarcoding of DNA extracts and a custom bioinformatics workflow identified potential incidence of species of concern which were subsequently validated through highly specific assays. Key genera, including Puccinia, Gremmeniella, Colletotrichum, and Alternaria, were investigated. This approach demonstrated the ability to screen hundreds of samples, while also providing a robust baseline dataset over five years for the development of an AI- based prediction tool. A graph neural network (GNN) is being developed to model the microbial communities (Amplicon Sequence Variants or ASVs from

metabarcoding data) and incorporate this data into a pathogen- incidence forecasting tool to identify possible new disease hotspots.

[O8] Next-Generation Sequencing of ITS regions for monitoring fungal pathogen infection and fungal community shifts in western redcedar

[Sydney Houston](#)^{1,2}, Jun-Jun Liu¹, Mike Cruickshank¹, Arezoo Zamany¹, Isabel Leal¹, Cosmin Filipescu¹, and Peter Constabel²

¹Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, 506 Burnside Rd W, Victoria, BC V8Z 1M5, Canada; ²University of Victoria, Forest Biology Department, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada

Correspondence to: sydney.houston@nrcan-rncan.gc.ca

Laminated root and butt rot, caused by the fungal pathogen *Coniferiporia weirii*, is a significant disease impacting western redcedar (WRC; *Thuja plicata*), an ecologically and economically important conifer native to North America. Despite its impact, little is known about *C. weirii* virulence or WRC resistance mechanisms. This study aimed to develop a reliable molecular detection and microbial profiling method to assess host susceptibility and evaluate fungal colonization in WRC fine root tissue. We utilized next-generation sequencing (NGS) of the fungal internal transcribed spacer (ITS) region to characterize the fungal communities associated with greenhouse WRC seedlings exposed to different treatments: inoculated with *C. weirii*, exposed but not inoculated (“control”), and completely isolated (true control). High-throughput sequencing allowed for comprehensive detection of fungal taxa, including successful identification of *C. weirii* ITS sequences in majority of inoculated seedlings. This detection pattern aligned with results obtained using a *C. weirii*-specific quantitative PCR assay. Relative abundance of *C. weirii* varied widely among individuals, suggesting variable infection or colonization success. Minimal detection was observed in control samples, while *C. weirii* was absent from all true control samples, confirming the specificity and effectiveness of the inoculation protocol. Additionally, sequencing data revealed distinct shifts in fungal community composition across treatments, indicating that *C. weirii* introduction has the potential to impact the WRC root mycobiome. Overall, ITS-based NGS proved to be an accurate, sensitive, and high-resolution method for detecting *C. weirii* and evaluating fungal community dynamics in WRC roots. This approach provides a valuable tool for understanding host-pathogen interactions and supports future research into WRC resistance mechanisms and forest health monitoring.

[O9] Genomic Detection and Monitoring of the Dutch Elm Disease

[Alice Y. Li](#)¹, Rory McIntosh², Carol Ritland¹, James Bush³, Joshua Pol², and Richard C. Hamelin¹

¹Department of Forest and Conservation Sciences, Faculty of Forestry, The University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada, V6T 1Z4; ²Forest Service Branch, Ministry of Environment, Government of Saskatchewan, 319 – 800 Central Avenue, Prince Albert, SK, Canada, S6V 6Z2; ³Crop Protection Laboratory, Ministry of Agriculture, Government of Saskatchewan, 1610 Park Street, Regina, SK, Canada, S4N 2G1

Correspondence to: richard.hamelin@ubc.ca

Dutch Elm Disease (DED) is the most destructive elm tree disease throughout North America and Europe caused by ascomycetes *Ophiostoma ulmi* and *O. novo-ulmi*. In Canada, DED is prevalent in most provinces east of Alberta, causing economic losses exceeding \$2.5B and reducing the ecological roles of elm trees as native species in urban and forest ecosystems. In summer 2024, it was discovered in Edmonton, Alberta and near Trail, British Columbia, marking the expansion of distribution and potential damage of the disease. Management efforts focus on monitoring and preventing the spread of the disease from infected areas. Early identification is essential for mitigation, especially on the western front of the pandemic. Current detection relies on visual symptoms, which are easily confused with less pathogenic *Plenodomus tracheiphilus*. Additionally, traditional laboratory identification requires 5 to 15 days for fungal culture. Our research aims to develop a rapid, on-site DNA detection tool for DED and to compare laboratory culture with field-ready qPCR and LAMP methods. Both qPCR and LAMP methods were tested during the 2024 DED season in Saskatchewan. To develop the multiplex qPCR primers for specificity and accuracy, we designed a marker assay for elm trees serving as an internal control. We are also testing other species-specific qPCR assays for the pathogen. Extraction protocol development and field performance validation will be conducted this year. We aim for this field detection tool to help in the early detection of DED and the prevention of its western spread into Alberta and British Columbia.

[O10] Isolation, pathogenicity and genetic diversity of *Verticillium longisporum* causing Verticillium stripe of canola on the Canadian Prairies

Longfei Wu¹, Yixiao Wang¹, Rudolph Fredua-Agyeman¹, Kan-fa Chang¹, Godfrey Chongo², Ahmed Abdelmagid³, Stephen E. Strelkov¹ & Sheau-Fang Hwang¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; ²BASF Canada Inc., Saskatoon, SK S7K 3J9; ³Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Rte 100 #100, Morden, MB R6M 1Y5
Correspondence to: sh20@ualberta.ca

Verticillium stripe, caused by *Verticillium longisporum*, is an emerging threat to *Brassica napus* (canola) production in Canada. First detected in Manitoba, the occurrence of *V. longisporum* has now been confirmed across multiple provinces. However, the genetic diversity and pathogenicity of *V. longisporum* populations in the Prairies remain largely unexplored due to the limited availability of pure cultures. To address this knowledge gap, a comprehensive survey was conducted in 2023 and 2024 across 75 fields in Alberta, 42 in Manitoba, and one in Saskatchewan. Canola plants exhibiting characteristic symptoms of Verticillium stripe were collected, yielding 143 purified fungal isolates morphologically identified as *Verticillium* spp., along with 48 additional isolates from Manitoba and Saskatchewan. Genomic DNA was extracted for species and lineage determination using two species-specific primer sets (*VeruniF2/VeruniR3* and *VlspF1/R4*) targeting the 18S rDNA intron region, as well as a multiplex PCR assay. Phylogenetic relationships were inferred by sequencing three loci: the internal transcribed spacer (ITS), beta-tubulin (TUB), and mitochondrial oxaloacetate transport protein (OX) genes. PCR and sequence analysis confirmed the predominance of *V. longisporum* lineage A1/D1, with three exceptions identified as *V. dahliae*.

Phylogenetic analysis revealed overall monophyly but detected genetic variability among isolates. Pathogenicity assays conducted *in vitro* and under controlled conditions demonstrated significant variation in virulence, with highly virulent isolates identified in Alberta compared to Manitoba. These findings highlight the comparable risk of *Verticillium* stripe across the Prairie provinces and underscore the need for further investigation into the epidemiology and management of this disease.

[O11] Starships and the evolution of virulence in tan spot (*Pyrenophora tritici-repentis*) of wheat

Reem Aboukhaddour^{1*}, Ryan Gourlie¹, Megan McDonald², Mohamed Hafez¹, Rexelle Asis¹, Rodrigo Ortega-Polo¹

¹Agriculture and Agri-Food Canada, Lethbridge, AB, Canada; ²School of Biosciences, University of Birmingham, Institute of Microbiology and Infection, Edgbaston, Birmingham, UK

Correspondence to: reem.aboukhaddour@agr.gc.ca

Starships are novel superfamily of transposable elements discovered recently in eukaryotes. They are defined by their large size up to 700 Kb, and by carrying combinations of cargo genes, including a tyrosine recombinase known as the captain gene, which is responsible for mediating their movement within and between species. Starships play a significant role in organismal evolution by promoting adaptation through gene duplication, disruption, and gain or loss of function. In my lab, we have used comparative analyses of long-read assembled genomes of the tan spot pathogen to demonstrate the involvement of starship elements in mobilizing virulence traits within this species. We provide evidence that *ToxA*, a necrosis- encoding effector, can be mobilized on different types of starships containing an intact tyrosine recombinase in various strains. Furthermore, we present novel evidence supporting the ancient mobilization of *ToxB*, a chlorosis-inducing effector, via starships that appear to be degraded, lacking the tyrosine recombinase yet still retaining other defining features of starships. These findings challenge the previous belief that *ToxB* was vertically inherited and instead highlight starships as powerful agents driving genome anatomy, pathogen adaptation, ecological flexibility, and the evolution of virulence in the tan spot pathogen, a major threat to wheat production worldwide.

[O12] Unraveling Effects of Anaerobic Soil Disinfestation on Soil Microbiomes and Strawberry Yield

Tika B. Adhikari¹, Ashley N. Philbrick¹, and Frank J. Louws^{1,2}

¹Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, United States; ²Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695, United States

Strawberry (*Fragaria × ananassa*) is a significant global horticultural crop. The application of anaerobic soil disinfestation (ASD) using organic amendments or readily available carbon sources can alter soil microbial communities, improve soil health, suppress soil-borne pathogens, and enhance crop productivity. Most research on the effects of ASD in strawberry cultivation has

concentrated on various regions in the U.S. However, there remain gaps in our understanding of how ASD impacts microbial communities and strawberry yields specifically in North Carolina. From 2019 to 2022, experiments were conducted at the Horticultural Crops Research Station in Castle Hayne to investigate the effects of ASD using dried molasses (5.60 tons/ha), mustard meal (2.24 tons/ha), a combination of both at half rates, a positive control (Pic Clor 60), and a negative control (no amendments) on soil bacterial communities and strawberry yields. Results from 16S amplicon sequencing showed significant differences in bacterial community composition over time, with higher alpha diversity observed in the organic amendment treatments compared to the controls. The key bacterial phyla identified include Proteobacteria and Actinobacteriota. All three ASD treatments increased the abundance of beneficial bacterial genera such as *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, and *Burkholderia*. The total marketable yield from the ASD treatments was comparable to that of the fumigation treatment. Overall, the study indicates that ASD with organic amendments positively affected both microbial community structure and strawberry yields, while fumigation reduced diversity, and untreated plots produced lower yields. This research highlights the potential of ASD to promote sustainability in strawberry cultivation in North Carolina.

[O13] Advancing *Plasmodiophora brassicae* genomics: single-cell sequencing reveals a contamination-free de novo assembly

Afsaneh Sedaghatkish¹, Meik Kunz², Bruce Gossen³, Mary Ruth McDonald¹

¹Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1; ²The Bioinformatics CRO, Inc. Sanford, FL United States, 32771; ³Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, SK, Canada, S7N 0X2

Correspondence to: mrmcdona@uoguelph.ca

Sequencing the whole genomes of unculturable microorganisms offers powerful insights into organisms that are difficult to study. In this study, we developed a cutting-edge pipeline for sample preparation and de novo assembly of *Plasmodiophora brassicae*, the obligate plant pathogen responsible for clubroot disease in brassica crops. This pathogen is genetically diverse, with numerous pathotypes identified. It can only complete its life cycle in a living host, which complicated previous genome sequencing efforts because of contamination from the host and soil microbes. To overcome this challenge, de novo genome assembly of *P. brassicae* was performed using sequences from ~4,000 single cells isolated from one root infected with a highly virulent pathotype. The assembly showed that it had an even distribution across chromosomes identified in previous assemblies, but with ~8% smaller chromosome sizes compared to the most recent reference genome. The total assembly size was 25.3 Mb, containing 8,758 genes. These findings suggest that ~8% (1.9 Mb) of the reference genome resulted from contamination by soil microbes. This new assembly represents a significant advancement in *P. brassicae* genomics by eliminating contaminants that may obscure factors such as virulence and resistance. Furthermore, the study highlights the potential of single-cell sequencing as a powerful tool for microbiological research, offering a new approach for studying non-culturable organisms.

[O14] Population Genomics of Clubroot Pathogen Reveals Its Diversity Dynamics and Evolution

Muhammad Asim Javed¹⁻⁴, Anne-Sophie Brochu¹⁻⁴, Elisa Ines Fontino¹⁻⁴, Florent Sylvestre¹⁻⁴, Soham Mukhopadhyay¹⁻⁴, and Edel Pérez-López¹⁻⁴

¹Département de Phytologie, Université Laval, Québec, QC, Canada, G1V 0A6; ²Centre de recherche et d'innovation sur les végétaux (CRIV), Université Laval, Québec, QC, Canada, G1V 0A6; ³Institute de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada, G1V 0A6; ⁴L'Institute EDS, Université Laval, Québec, QC, Canada, G1V 0A6.

Correspondence to: edel.perez-lopez@fsaa.ulaval.ca

Plasmodiophora brassicae, the causal agent of clubroot, threatens the global brassica industry, including Canada's \$43 billion canola sector. Clubroot-resistant (CR) cultivars are the primary management strategy but drive pathogen evolution, leading to resistance breakdown. Despite its importance, the global population genomics of *P. brassicae* remains largely unexplored. In this study, we sequenced the whole genome of 134 *P. brassicae* isolates from diverse Brassica hosts across 24 countries from five continents. Our analysis identified three genetic clusters: one comprising western Canadian populations, a second with international isolates, and a third, more scattered cluster with genetically distinct Quebec isolates. A genome-wide scan of candidate effectors revealed a clustering pattern aligned with population structure, along with highly polymorphic putative effectors that may drive pathogen adaptation. To resolve genomic variation, we performed a pangenome analysis using 47 high-quality genome assemblies from long-read sequencing, representing the three genetic clusters, diverse geographic origins, and varying virulence profiles. The *P. brassicae* pangenome spans 29.4 Mb, capturing ~4 Mb of total genomic variation despite a highly conserved core genome. This first global genomic analysis of *P. brassicae* reveals key genetic and structural variations shaping its evolution. The high-resolution pangenome uncovers polymorphic effectors linked to CR resistance breakdown, offering critical insights for breeding durable resistance and improving clubroot disease management.

[O15] Developing Heterologous Expression of Rust Effectors in a Necrotrophic Fungal Pathogen

Md Al Mamun, Zhaohui Liu, Malaika Ebert and Upinder Gill

Department of Plant Pathology, North Dakota State University, Fargo, ND, USA

Correspondence to: md.almamun@ndsu.edu

Plant-Pathogen interactions follow the gene-for-gene model for biotrophic pathogens, where plant resistance genes (R) encode for immune receptors that recognize corresponding products of pathogen avirulence genes to trigger defense responses, often leading to programmed cell death (PCD) and resistance. However, in a necrotrophic pathosystem, dominant host sensitivity gene(s) recognizes the corresponding necrotrophic effector to induce extensive cell death (necrosis) leading to susceptibility. Rust pathogens are obligate biotrophs and it is nearly impossible to functionally validate avirulence effectors in pathogen itself. In this study, we aim to establish a unique method to functionally characterize wheat rust avirulence effectors via expressing them in

the wheat necrotrophic pathogen *Parastagonospora nodorum*. To prove this concept, two known rust effector genes, *AvrSr35* and *AvrSr50* were individually transformed into *P. nodorum* and the culture filtrates of the successful transformants were infiltrated into wheat lines carrying individual resistance genes *Sr35* and *Sr50*, respectively. However, no necrotic reactions were observed for both infiltrations. The RT-PCR showed the expression of *AvrSr35* and *AvrSr50* in *P. nodorum*. Western blotting is being performed to check if rust effectors are produced and properly secreted in fungal cultures. The possible challenge of using necrotrophic fungi to express rust effectors will be discussed.

[O16] Genomic diversity of Tomato brown rugose fruit virus in Canadian greenhouse production systems

Jonathan S. Griffiths¹, Gregory Fougere^{1,2}, Dong Xu³, Cara McCreary⁴, Genevieve Marchand⁵, Aiming Wang⁶, and Mamadou Fall Lamine³

¹London Research and Development Centre, Agriculture and Agri-Food Canada, 4902 Victoria Ave N, Vineland Station, ON L0R 2E0, Canada; ²Department of Biological Sciences, Brock University, St. Catharines, ON L2S 3A1, Canada; ³St. Jean sur Richelieu research and development center, Agriculture and Agri-Food Canada; ⁴Ontario Ministry of Food and Rural Affairs; ⁵Harrow Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, N5V 4T3, Canada; ⁶London Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, N5V 4T3, Canada

Tomato brown rugose fruit virus (ToBRFV) is a recently emerged viral pathogen in the Tobamovirus genus. ToBRFV was first observed in 2014 in the middle east, and has since spread worldwide causing significant losses in greenhouse tomato production. ToBRFV can escape the durable *Tm-2²* resistance gene, facilitating its worldwide spread. A number of new resources of resistance have been reported but the identity, number, and mechanisms of these putative novel resistance genes is largely unknown. Here we report 12 novel ToBRFV genomic sequences from Canadian greenhouse production systems, in susceptible and resistant varieties since 2023, and combined these sequences with five other Canadian ToBRFV genomes previously deposited on Genbank. At least three groupings of sequences were identified, suggesting multiple introductions of ToBRFV into Canada. Analysis of the dN/dS ratio of the four ToBRFV ORFs suggest that p126 and the MP are under negative or purifying selection. A number of novel non-synonymous substitutions in the p126 and Movement Protein (MP) ORFs were identified unique to Canadian isolates, and associated with novel resistant tomato varieties. Polymorphisms in the p126 ORF are located in a region of the protein associated with *Tm-1* resistance breaking isolates of tomato mosaic virus. Five novel polymorphisms in MP were also identified and could be related to other novel avirulence genes. Together, these results suggest multiple introductions of ToBRFV into Canadian tomato production systems, identify putative adaptations to novel tolerance genes, and emphasize the urgent need for the cloning and characterization of novel sources of resistance to ToBRFV.

[O17] FROM Genome to Pathotyping: Exploring Comparative Genomics for Plasmodiophora brassicae

Sandra M. Velasco-Cuervo¹, Yoann Aigu¹, Leonardo Galindo-González^{1,2}, Sheau-Fang Hwang¹, and Stephen E. Strelkov¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5; ²Ottawa Plant Laboratory (Fallowfield), Science Branch, Canadian Food Inspection Agency, Ottawa, ON K2J 4S1

Correspondence to: sandrarama@ualberta.ca

Plasmodiophora brassicae causes clubroot, a soilborne disease of canola (*Brassica napus*) and other crucifers. Pathogen isolates are currently classified into pathotypes based on virulence phenotypes using host differential sets, a process requiring biosecure greenhouse facilities and up to eight weeks. Metabarcoding presents a promising alternative for rapid pathotype identification, enabling the processing of hundreds of samples within days once standardized. To support this approach, we conducted a comparative genomics analysis to develop high-quality genome assemblies for key Canadian *P. brassicae* pathotypes. High-fidelity sequencing was used to generate long reads and construct *de novo* whole-genome assemblies for single-spore isolates representing seven pathotypes. These assemblies exhibited an average completeness of 80.84%, as assessed by BUSCO analysis, with a mean genome size of 24.55 Mb. Genome annotation revealed that repetitive regions constituted approximately 30% of the genome, with an average of 11,418 coding sequences per assembly. Mitochondrial genomes average 114,084 bp with 32 protein-coding genes, 28 structural RNA genes, and 27 open-reading frames. Pangenome analysis showed that the core genome accounted for 66.07% of the total genome length, while the accessory genome comprised the remaining 33.92%. Comparative analysis identified 981 structural variants and around 60,000 single nucleotide polymorphisms across the assemblies. Laboratory experiments are currently underway to validate polymorphic regions for potential integration into a metabarcoding assay for pathotype detection. Additionally, this study enhances our understanding of *P. brassicae* evolution and genomic architecture.

[O18] Unraveling Microbial Defenses: Metagenomics-Guided Discovery of Plant Growth-Promoting Bacteria for Potato Wart Suppression

Ishraq Akbar^{1,2}, Yichao Shi¹, Bart T.L.H. van de Vossen³, Theo A.J. van der Lee¹⁴, Linda E. Jewell⁵, Sean Li⁶, Hai D.T. Nguyen¹, Wen Chen^{1,2}

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, Canada, K1A 0C6; ²Department of Biology, University of Ottawa, Marie-Curie Private, Ottawa, ON, Canada, K1N 9A4; ³Dutch National Plant Protection Organization, Geertjesweg 15, 6706EA Wageningen, the Netherlands; ⁴Wageningen University & Research, Droevendaalsesteeg 4, 6708 PB Wageningen, the Netherlands; ⁵St. John's Research and Development Centre, 308 Brookfield Road, St. John's, NL, Canada, A1E 6J5; ⁶CFIA Laboratory, 93 Mt Edward Rd, Charlottetown, Canada, PE C1A 5T1

Correspondence to: wen.chen@agr.gc.ca

Potato wart is a devastating soil-borne disease caused by the obligate biotrophic chytrid *Synchytrium endobioticum*, a quarantine pathogen in Canada and a select agent in the U.S. Current containment relies on strict phytosanitary measures and planting resistant varieties of potatoes, but both face challenges related to cost and long-term effectiveness. Phytomicrobiomes harbor plant growth-promoting bacteria (PGPB) that can induce systemic resistance, promote fungistasis, and contribute to disease suppressiveness, making them a reservoir of potential biocontrol agents against potato wart. To characterize the soil and wart microbiomes, we obtained ~200 environmental DNA samples from the Netherlands and Canada (PEI and Newfoundland). In a pilot study, 61 samples from the Netherlands (28 diseased, 33 healthy) underwent whole genome amplification and full-length 16S rRNA sequencing on the Nanopore MinION platform. Metabarcoding sequences were processed using the Apogee pipeline, generating 37,462 Amplicon Sequence Variants (ASVs), with taxonomic classification assigned against the SILVA database. Of these, 3,874 ASVs were identified as potential PGPB based on an in-house literature-curated database. Co-occurrence network analysis revealed microbial interactions potentially linked to *S. endobioticum* suppression. Significant differences in community composition and function were observed between wart and infested tare soils, as well as between sieve fractions (75 µm vs. 25 µm). These findings provide the first insights into wart-associated microbiome and highlight candidate PGPB for biocontrol development.

[O19] Cinderella slipper? Examining Host Range expansion and Spore Specificity in *Cronartium ribicola*, the Causal Agent of White Pine Blister Rust

B.P. DUARTE¹ R. C. HAMELIN¹

¹Department of Forest & Conservation Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada

Correspondence to: brian.duarte@ubc.ca

Rust fungi (*Pucciniales*) are highly complex plant pathogens capable of producing up to five unique spore types. Heteroecious species, such as the invasive *Cronartium ribicola* J.C. Fisch—the causal agent of white pine blister rust—produce specialized spore types to infect unrelated host species. Basidiospores infect aecial hosts, specifically *Pinus* species in the *Strobos* subgenus, while aeciospores and urediniospores infect telial hosts within the flowering plant clades *Ribes* and *Orobanchaceae*. Questions have been raised about how spore specificity is maintained and if *C. ribicola*'s host range is truly limited to these two angiosperm clades or if this assumption stems from a lack of testing and monitoring. Experimental inoculations on several North American angiosperm families revealed new compatible hosts within the *Mentzelia* genus. The susceptibility and severity of infection in *Mentzelia* plants were compared to the highly susceptible and well-known host *Ribes nigrum*. Further inoculation tests were done to reveal how different spore types recognise their compatible hosts compared to their alternate incompatible hosts. comparisons were made with infected telial hosts and infected western white pine to uncover host-specific gene expression underlying host alternation. These findings provide valuable insights into the evolution of host specificity, pathogen adaptability, the possible development of new races specialized for different alternate hosts, and the role of host jumps in rust speciation. Further

concerns arise from *C. ribicola*'s ability to exploit a broader host range, potentially enabling the pathogen to spread into new environments and higher altitudes, thereby increasing the risk to susceptible white pines.

[O20] A protein kinase fusion gene from *Aegilops speltoides* provides broad resistance against wheat stem rust

Jason D. Zurn^{1,2}, Ural Yunusbaev^{1,2}, Yuanwen Guo², Fei He², Wei Wang^{1,2}, Andres Salcedo², Monica Fernandez De Soto^{2,3}, John Fellers⁴, István Molnár⁵, Jaroslav Dolezel⁵, Steven Xu⁶, Alina Akhunova^{2,3}, Harold Trick², Matthew Rouse⁷, Eduard Akhunov^{1,2}

¹Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA; ²Department of Plant Pathology, Kansas State University, Manhattan, KS, USA; ³Integrated Genomics Facility, Kansas State University, Manhattan, KS, USA; ⁴USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS, USA; ⁵Institute of Experimental Botany, Olomouc, Czech Republic; ⁶USDA ARS, Western Regional Research Center, Albany, CA, USA; ⁷USDA-ARS, Cereal Disease Lab, St. Paul, MN, USA.

Correspondence to: eakhunov@ksu.edu

Wild wheat relatives have been a rich source of novel resistance (R) genes, protecting the world's wheat crop against diverse pathogen threats. Two such R genes, originating from *Aegilops speltoides*, include the stem rust R genes *Sr39* and *Sr32*. To map and characterize these genes we employed a multi-faceted approach including microscopy, whole-genome sequencing (WGS), mutagenesis, and transgenic complementation. These R genes encode allelic variants of an ancient fusion between a protein kinase domain and the CBP60-like DNA-binding domain (*PKCL1*), both of which are required for resistance. Upon infection PKCL1 accumulates in the nucleus, consistent with the presence of nuclear localization signals, and provides broad resistance to Pgt. Synteny analysis revealed the PKCL1 domain fusion was lost in the B-genome ancestor of wheat and other Sitopsis species but was retained in *A. speltoides*. WGS of 30 diverse *A. speltoides* accessions revealed likely-functional allelic variants of PKCL1. Finally, transcriptomic and phytohormone analyses of infected wild-type and *pkcl1* mutants suggest salicylic acid-dependent pathways are likely associated with the *PKCL1*-mediated response to *Puccinia graminis* f. sp. *tritici*. To date, much work has been done towards understanding NLR-mediated wheat rust resistance, however little work has been done towards exploring a new class of R genes consisting of protein kinases fused to other domains. *PKCL1* represents another exciting opportunity to further explore protein kinase fusion genes and unravel the complexities surrounding disease resistance mechanisms mediated by R genes with diverse domain architectures.

[O21] Expanding Wheat's Genetic Horizon: Wild Germplasm as a Source of Biotic and Abiotic Stress Resistance

Satinder Kaur¹, Mhatre Dhanashree Prashant¹, Bukke Kutti Bai¹, Tanu Shri¹, Pooja Rani¹, Safoura Shafi¹, Kuldeep Singh² and Parveen Chhuneja¹

¹School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004;

²International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, Telangana 502324

Correspondence to: satinder.biotech@pau.edu

Genetic advancements have significantly enhanced wheat productivity, improving resilience to biotic and abiotic stresses. However, climate change and emerging pathotypes demand robust genetic resources for sustainable wheat production. Wild relatives of wheat, including *Triticum* and *Aegilops* species, hold invaluable genetic diversity, yet remain largely underutilized. At Punjab Agricultural University, Ludhiana, we maintain over 1,200 wild wheat accessions belonging to 22 species and diverse material introgressed from these accessions. These wild species serve as a goldmine of novel alleles, with single accessions often harboring genes for multiple traits. Even single wild chromosomes carry multiple beneficial alleles, offering a powerful resource for wheat improvement. We have successfully introgressed rust resistance genes from wild species, including *Ae. triuncialis*, *Ae. kotschyii*, *Ae. peregrina*, *Ae. speltoides*, *Ae. lorentii*, and *T. monococcum*. Genomic mapping revealed that many introgressed lines not only exhibit resistance to both leaf and stripe rusts but some having resistance to powdery mildew and karnal bunt. We identified and mapped multiple QTLs for resistance to fusarium head blight, leaf blight, and lesser grain borer, along with heat stress tolerance and improved nitrogen use efficiency from an in-house developed synthetic wheat (*T. durum* × *Ae. tauschii*, accession 14135). Similarly single 5U chromosome from *Ae. triuncialis* in a hexaploid wheat background harbors QTLs for resistance to powdery mildew & cereal cyst nematode and to drought tolerance. Strategic introgression, coupled with advanced genomics, provides a powerful approach to enhancing wheat resilience and productivity in the face of global agricultural challenges.

[O22] Utilization of Wheat Wild Relatives to Improve Wheat Yield and Rust Resistance

Zhen Wang^{1,5}, Kyle Parker^{1,5}, Zhiwu Zhang², Eduard Akhunov³, Paul St Amend⁴, Guihua Bai⁴, Qingwu Xue⁵, Jackie C. Rudd⁵, Amir M. H. Ibrahim¹, Dirk B. Hays¹, Shannon Baker⁵, Jason Baker⁵, Geraldine B. Opena¹, Daniel Hathcoat, Bryan E. Simoneaux¹, Russell Sutton¹, Shuyu Liu^{1,5}

¹Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, United States of America; ²Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, United States of America; ³Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, United States of America; ⁴USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, United States of America; ⁵Texas A&M AgriLife Research, 6500 Amarillo Blvd W, Amarillo, TX 79106, United States of America.

Correspondence to: shuyu.liu@ag.tamu.edu

Primary synthetic is one of the major resources that has been utilized in spring wheat breeding worldwide to improve drought tolerance and pest resistance but little research has been conducted in winter wheat backgrounds. Texas A&M Wheat breeding and genetic programs have utilized synthetic hexaploid wheat to introduce novel alleles from various tetraploid durum wheat (*Triticum turgidum* L.) and diploid tauschii (*Aegilops tauschii* L.) into the hard red winter wheat for more than 20 years. In this study, two adapted popular wheat cultivars TAM 111 and TAM 112 were designated as recurrent parents and backcrossed with 21 synthetic hexaploid wheat lines, then those derived lines were advanced for five generations to generate a set of 298 BC₁F_{5.7} synthetic

derived wheat lines (SDLs) as an advanced-backcross nested association mapping population (AB-NAM). Genotyping-by-sequencing (GBS) and skim-seq were used to impute a large set of 28 million single nucleotide polymorphisms (SNPs) and insertion deletions (Indels) from parental whole genome sequencing (WGS) data. Yield, quality, rust, insect resistance traits of the population was evaluated in 13 environments across Texas for genome-wide association analyses to identify significant SNPs and performing genomic selections. These developed SDLs provided novel genetic resources and broadened the winter bread wheat gene pool. Significant SNPs linked to target beneficial alleles were used to explore the efficient utilization of synthetic wheat in breeding programs. In addition, genomic prediction with multiple traits/environments/models provided in-depth knowledge for the breeding application of these SDLs.

[O23] Uncovering Yellow Rust Resistance Loci in Watkins Wheat Landraces via GWAS

Jasneet Singh¹, Samuel Holden¹, Rajdeep S. Khangura¹ and Gurcharn Singh Brar¹

¹Faculty of Agricultural, Life, and Environmental Sciences, University of Alberta, Edmonton, AB, Canada

Correspondence to: gurcharn@ualberta.ca

Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, severely impacts global wheat production, compromising yields and quality. The A.E. Watkins wheat collection, comprising 827 diverse landraces from 32 countries, provides a valuable genetic reservoir for enhancing resistance. We performed a genome-wide association study on 300 Watkins lines to uncover quantitative trait loci (QTLs) conferring resistance to six yellow rust strains: W034, W001, T022, W056, W031, and W057. Pearson correlation analysis among the strains showed moderate to strong positive associations, with W031 and W001 having the highest overlap, minimal negative correlations, and some independence, especially between T022 and W056, indicating diverse resistance mechanisms. Disease score distributions showed moderate to high susceptibility (medians 5-7), with W031 and W056 showing the greatest severity. Genotypic data, sourced from Watkins & Worldwide Wheat G2B, were filtered for minor allele frequency (0.02), genotyping rate (0.1), individual missingness (0.1), and Hardy-Weinberg equilibrium ($1e-10$), yielding 209,722 SNPs. Phylogenetic analysis of these SNPs resolved the lines into eight major groups, reflecting their genetic diversity. Using a mixed linear model, we identified 13 significant QTLs across multiple chromosomes. For strain W031, four QTLs were detected on chromosomes 2A, 5B, 6B, and 6D. Strain W056 revealed three QTLs on 3A and 5B, while W057 identified four QTLs on 2B and 5B. Strain W034 showed two QTLs on 7A. These findings highlight novel resistance loci, advancing our knowledge of yellow rust resistance. Future work aims to characterize candidate genes within these QTLs, exploring both novel and known yellow rust resistance genes to inform marker-assisted breeding for resilient wheat varieties.

[O24] Azoles vs. DMIs: A divide in fungicide resistance research terminology

Edward McNab¹, and Tom Hsiang¹

¹University of Guelph, School of Environmental Sciences. 50 Stone Rd E, Guelph, ON, Canada, N1G 2W1

Correspondence to: thsiang@uoguelph.ca

Azole fungicides, also known as demethylation inhibitors (DMIs), are a key tool in managing fungal pathogens in phytopathology as well as human/animal medicine. Despite their abundant use, differences in terminologies and methodologies have led to a divide in the research on fungicide resistance. For example, in phytopathological research, the target enzyme is typically designated CYP51, whereas literature on human/animal pathogens refers to it as Erg11, which is the same thing. This discrepancy is compounded by differences in resistance metrics, with phytopathologists relying on effective concentration (EC₅₀) values, and human/animal medical research using minimum inhibitory concentrations (MIC). Additionally, resistance mechanisms, including point mutations, efflux pump overexpression, and biofilm formation, are documented in both sectors but are rarely compared between them. Variations in typical application methods, such as topical treatments in plants versus systemic injections or oral intake in humans/animals, may also influence the evolution of resistance and its mechanisms. This research provides an overview of the historical and methodological factors that may have led to these differences, and explores their implications for resistance monitoring and management. We encourage interdisciplinary collaboration with harmonized nomenclature (e.g., dual CYP51/Erg11 designation) and standardized evaluation protocols, alongside the curation of collaborative databases, such as the recent FungAMR database, which provides a listing of reported fungicide resistance literature and mechanisms from 462 published articles. By integrating perspectives through “One Health” strategies, the proposed efforts will benefit resistance tracking, monitoring, and management, to provide insight into fungicide resistance, and ultimately help safeguard both crop productivity and human/animal health.

[O25] Outcomes of competitive dynamics of *Fusarium graminearum* and *F. poae* on wheat heads

Imane Laraba¹, Briana K. Whitaker², Susan P. McCormick², Christina Cowger^{3,4}, Peter Oppenheimer³, and Martha M. Vaughan²

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, Canada, K1A 0C6; ²Mycotoxin Prevention and Applied Microbiology Unit, National Center for Agricultural Utilization Research, United States Department of Agriculture, 1815 University Street, Peoria, IL, USA, 61614; ³Department of Entomology and Plant Pathology, North Carolina State University, 1575 Varsity Drive, Raleigh, NC, USA, 27695; ⁴Plant Science Research Unit, United States Department of Agriculture, 1575 Varsity Drive, Raleigh, NC, USA, 27695

Correspondence to: Imane.laraba@agr.gc.ca

Although *Fusarium graminearum* (*Fg*) is the pathogen responsible for most *Fusarium* head blight (FHB) outbreaks on wheat in North America, several other weaker pathogens (e.g., *F. poae* [*Fp*]) co-exist within the same head or field in certain agroclimatic settings. The implications of the competitive dynamics between *Fg* and *Fp* within the same head for FHB development and

mycotoxin contamination remain poorly understood. Here, we evaluated whether pre-inoculation with *Fp* two days prior to *Fg* or co-inoculation of the two pathogens would impact *Fg* disease outcomes. We hypothesized that disease outcomes would be dependent on 1) the *Fg* population/chemotype (NA1/15-ADON and NA2/3-ADON), 2) the wheat variety (Alsen and Norm), and 3) wheat immune responses. Our preliminary results indicate pre- and co-inoculation of *Fp* with both NA1 and NA2 *Fg* strains resulted in significantly greater disease severity and deoxynivalenol (DON) toxin accumulation on cultivar Alsen heads compared to treatments of *Fg* alone. Similarly, on cultivar Norm heads, pre- and co-inoculation of *Fp* with the NA2 *Fg* strain caused higher FHB severity and DON contamination relative to NA2 *Fg* reference inoculation. For the NA1 strain treatments, no consistent statistically significant difference was noted in disease development and DON accumulation between pre- or co-inoculated heads as compared to the reference treatments of *Fg* strain inoculated alone. Analyses assessing the level of the plant defense phytohormones salicylic and jasmonic acids and expression patterns of their biosynthetic genes are underway to gain insight into the mechanisms underlying the observed disease outcome differences.

[O26] Genetic mapping of aggressiveness in *Fusarium graminearum*, causing Fusarium head blight in durum wheat

Yishan Zhang^{1,2}, Gurcharn S. Brar^{2,3}, and Ehsan Sari⁴

¹Michael Smith Laboratories, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada;

²Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC V6T 1Z4,

Canada; ³Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton,

AB T6G 2P5, Canada; ⁴Department of Microbiology and Plant Pathology, University of California Riverside, Riverside, CA 92521, USA

Correspondence to: zyscedar@mail.ubc.ca

Fusarium graminearum, the predominant causal agent of Fusarium head blight (FHB) in cereal crops, impacts the Canadian wheat industry through reducing grade and yield, and seed contamination with mycotoxins. Depending on the type of mycotoxins produced by *F. graminearum*, strains can be classified into multiple chemotypes, including 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON). A highly virulent population of the 3ADON chemotype is becoming more prevalent in North America, compared to the historically dominant population which produces 15ADON, resulting in an increasing risk of FHB in the Canadian wheat growing regions. Therefore, decoding the genetic factors of aggressiveness and their evolution among field populations is crucial for studying the wheat-Fusarium interactions. This project aims to map quantitative trait loci (QTLs) conferring aggressiveness in *F. graminearum* (Fg-QTL), using a bi-parental population Fg09 (n = 170) derived from a cross between a 15ADON isolate (Nit5) and a 3ADON isolate from Saskatchewan (SK1797). A high-density genetic map was constructed and aggressiveness on the durum cultivar Kronos was evaluated. Through pan-genome analysis of a Nested Association Mapping Population (FgNAM), previously constructed to reflect aggressiveness variations in North American field populations, two Fg-QTLs were identified,

and one of them locates in a sub-telomeric co-occurring with a genomic region with high nucleotide diversity on chromosome 3. This Fg-QTL explained 8.18 % of the variance in the area under disease progress curve (AUDPC) values and contained 36 genes. Three candidate genes were detected as potentially associated with the observed phenotypic differences in aggressiveness, as they exclusively held nucleotide variants unique to high-aggressiveness strains. Two of the genes encode proteins with predicted functions, mic19 and FLA protein, provided promising targets for further validation of the genetic basis of *F. graminearum* aggressiveness on durum wheat.

[O27] A Stripe Rust Pan-Genome Provides Evidence for Ongoing Somatic Hybridisation in Nature

Samuel Holden^{1,2}, Meng Li¹, Mehrdad Abbasi¹, Ramandeep Bamrah¹, Sang Hu Kim³, Sean Formby³, Sean Walkowiak⁴, Upinder S. Gill⁵, Guus Bakkeren³, Curtis J. Pozniak⁶, Gurcharn S. Brar^{1,2}

¹Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada; ²Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; ³Agriculture and Agri-Food Canada, Summerland Research Center, BC, V0H 1Z0, Canada; ⁴Canadian Grain Commission, Grain Research Laboratory, MB, R3C 3G8, Canada; ⁵North Dakota State University, Fargo, ND, USA; ⁶Crop Development Centre, University of Saskatchewan, Saskatoon, SK S7N 5A8 Canada

Correspondence to: Sholden1@ualberta.ca

When considering the wheat rust diseases caused by *Puccinia striiformis*, *Puccinia triticina*, and *P. graminis*; a critical concern is the emergence of new, highly virulent pathogen lineages as exemplified by the 2015 Warrior epidemic, or the ongoing spread of the Ug99 stem rust lineage. Advances in sequencing technology have made it viable to monitor rust populations through whole genome sequencing of field isolates. We assemble high-quality PacBio HiFi genomes of over 20 North American isolates of *Puccinia striiformis* f. sp. *tritici* (*Pst*) and three isolates of *P. striiformis* f. sp. *hordei* (*Psh*); developing a comprehensive pan-genome of *P. striiformis*. We compare two haplophased genomes representing the major *Pst* lineages present in North America: *PstS1* and *PstS18*. We demonstrate that the dominant *PstS18* lineage shares a single haploid genome with the older *PstS1* lineage, with no evidence of recombination or reassortment. We hypothesize that the lineages are therefore related through somatic hybridization with a third, unknown, lineage. We also utilise a unique “pseudo-phasing” approach, in which members of a clonal lineage are assumed to undergo minimal recombination, to characterise the effects of somatic hybridisation on gene content. This identified a third lineage “*PstS18-2*” which shows high aggressiveness and spore production on Canadian wheat lines and may have originated in an independent hybridisation event. To our knowledge this marks the third demonstration of lineage emergence via somatic hybridization in the wheat rusts and the first in *Pst*. This result underscores the role of somatic hybridisation in *Pst* evolution and lineage emergence worldwide.

[O28] Technical Challenges and Approaches in Constructing Nuclei-Phased Genome Assemblies of dikaryotic *Puccinia triticina* Using Early Noisy Long-Read Datasets

Sean Formby^{1,2}, Sang Hu Kim¹, Rob Lining¹, Samuel Holden³, Gurcharn S. Brar³, Steven J. Hallam^{2,4}, John Fellers⁵, Guus Bakkeren¹

¹Agriculture and Agri-Food Canada, Summerland Research Center, BC, VOH 1Z0, Canada;

²Graduate Program in Bioinformatics, Faculty of Science, The University of British Columbia, 570 West 7th Avenue, Vancouver, BC, V6T 1Z4, Canada; ³Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; ⁴Department of Microbiology and Immunology, Faculty of Science, The University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC, V6T 1Z3, Canada; ⁵USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, USA

Correspondence to: sean.formby@alumni.ubc.ca

The wheat leaf rust pathogen, *Puccinia triticina* (*Pt*), is an obligate biotrophic fungus with a complex dikaryotic genome, characterized by extensive heterozygosity and repetitive content. These features have historically made phased genome assembly challenging, particularly before the advent of high-accuracy long-read sequencing. While newer technologies such as PacBio HiFi and Oxford Nanopore (ONT) duplex sequencing, combined with Hi-C chromatin conformation capture, now enable high-quality, nuclei-phased assemblies, these approaches can be costly and some isolates of interest have already been sequenced with earlier, noisier long-read technologies creating challenges for comparative analysis. Here, we present a genome assembly pipeline that reconstructs phased dikaryotic genomes from early-generation PacBio and ONT datasets. By incorporating iterative assembly refinements and graph-based phasing approaches, we reconstructed phased genome assemblies on the chromosomal level and at nuclei resolution. To assess assembly and phasing accuracy, we employed multiple biological and technical benchmark features, including expected haplotype specific structural variants, telomeric motifs, synteny conservation with high-fidelity assemblies and comparisons to a large population-based phased panel. The resulting workflow and benchmark feature set demonstrates that even with higher error-rate long-read data, phased assemblies of *Pt* can be achieved with structural accuracy comparable to those generated with newer sequencing technologies. This can provide a cost-effective alternative to resequencing and highlights bioinformatic strategies that can maximize the use of older datasets enabling more robust comparative analysis adaptable to many dikaryotic/diploid organisms. In the context of the current study, this will allow for the generation of phased gene allele compendiums in the *Pt* pan-genome, particularly for effectors, improving our understanding of plant pathogen evolution.

[O29] Haplotype-phased genomes reveal the evolutionary history of race groups TKTTF, TTRTF and avirulence gene variants associated with severe wheat stem rust epidemics

Rebecca E Spanner¹, Eva C Henningsen², Feng Li¹, Oadi Matny¹, David P. Hodson³, Nino Virzi⁴, Kim-Phuong Nguyen⁴, Jibril Lubega⁶, Kostya Kanyuka⁶, Matthew Moscou⁵, Zacharias A. Pretorius⁷, Willem Boshoff⁷, Jana Sperschneider⁸, Peter Dodds⁸, Brian Steffenson¹, Melania Figueroa⁸

¹University of Minnesota, St. Paul, MN, U.S.A. 55108; ²Research School of Biology, The Australian National University, Canberra, ACT, Australia 2601; ³International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico 56237; ⁴Centro di Ricerca Cerealicoltura e Colture Industriali

(Cl), Acireale, CT, Italy 95024; ⁵Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A. 55108; ⁶NIAB, Cambridge, United Kingdom CB24 9NZ; ⁷University of the Free State, Bloemfontein, South Africa 9300; ⁸Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT, Australia 2601

Correspondence to: Melania Figueroa (melania.figueroa@csiro.au) and Peter N Dodds (peter.dodds@csiro.au)

A recent milestone in dikaryotic rust (*Puccinia*) genomics has been the development of haplotype-phased genomes. This has enabled tracking of entire nuclear haplotypes to reveal evidence of somatic hybridisation events leading to the emergence of novel race groups (e.g. TTKSK/Ug99). For their roles in recent severe wheat epidemics and virulence on widely deployed R genes, we performed PacBio HiFi and Hi-C sequencing for isolates ETH2013-1 and ITA2018-1. ETH2013-1 is of race group TKTF which dominated during the severe 2013-2014 epidemic on wheat cultivar Digalu after overcoming *SrTmp*. ITA2018-1 is of race TTRTF which overcame *Sr13b* and *Sr35* causing severe epidemics on durum wheat in Sicily, Italy during 2016-17. The phased nuclear genomes revealed novel nuclear haplotypes G and H in ETH2013-1 and haplotypes I and J in ITA2018-1. The TKTF race group is genetically diverse: the first described TKTF “type” strain 13ETH18-1 (Olivera *et al.* 2015, *Ecol. Epidemiol.* 105:917-928) is in Clade IV-A and is genetically distinct from ETH2013-1 (Clade IV-B). K-mer containment analysis of Illumina reads revealed that German and Georgian isolates in Clade IV-C contain haplotype H of ETH2013-1, but not haplotype G, suggesting a historical nuclear exchange event. Novel variants for known avirulence effectors (*AvrSr13*, *AvrSr22*, *AvrSr27*, *AvrSr35* and *AvrSr50*) and candidates for additional avirulence effectors were mined and tested for recognition using functional assays. We have established novel allele nomenclature for an *AvrSr* gene atlas comprised of functionally characterized *AvrSr* variants which can be used to determine the virulence of surveyed isolates on deployed *Sr* genes.

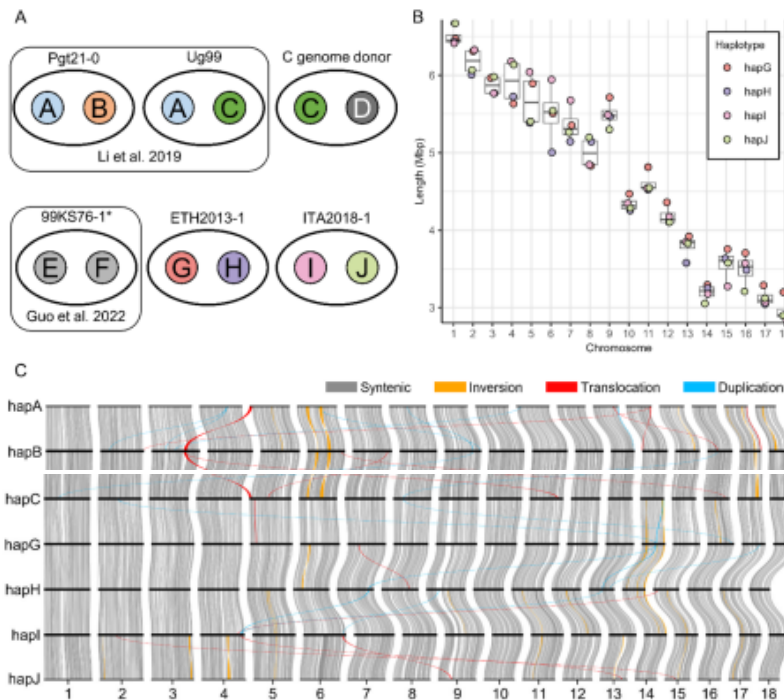


Figure 1. a) Nuclear haplotype designations for *P. graminis* f. sp. *tritici* isolates. Asterisk indicates that available reference haplotypes are not nuclear phased. **b)** Boxplots of chromosome lengths for haplotypes from ETH2013-1 and ITA2018-1. Box boundaries represent the first and third quartile and the center line represents the median length. Colors represent nuclear haplotypes. **c)** Syntenic plots showing syntenic regions and incidences of inversion, translocation and duplication after pairwise alignments between haplotypes.

[O30] Wheat leaf rust pathogen genomics

Guus Bakkeren¹, Sean Formby², Sang Hu Kim¹, Samuel Holden³, Janesse Holmes¹, Lu Liu¹, Meng Li⁴, Mei-Lan de Graaf¹, Rob Linning¹, Xiben Wang⁵, Randy Kutcher⁶, Gurcharn Singh Brar³, Brent McCallum⁵, John Fellers⁷

¹Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, BC, V0H1Z0, Canada; ²Bioinformatics Program, Michael Smith Laboratories, Faculty of Science, University of British Columbia, 6200 University Blvd. Vancouver, BC, V6T1Z4, Canada; ³Faculty of Agricultural, Life & Environmental Sciences, The University of Alberta, Edmonton, T6G1C9, Canada; ⁴Department of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T1Z4, Canada; ⁵Agriculture and Agri-Food Canada, Morden Research and Development Centre, Morden, MB, R6M1Y5, Canada; ⁶Crop Development Centre/Department of Plant Science, University of Saskatchewan, Saskatoon, SK, S7N5A8, Canada; ⁷USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, USA

Correspondence to: guus.bakkeren@agr.gc.ca

Puccinia triticina is an obligate basidiomycete pathogen causing leaf rust of wheat and an omnipresent worldwide scourge resulting in significant crop losses in many regions annually. Studying

this fungus on a molecular genetic level is challenging since it cannot be grown in culture and many tools commonly used for other organisms are unavailable. However, the genomics era has produced ever-growing resources that in a major way have advanced insight into the biology and the evolution of the populations of this fungus, as well as the interaction with its host including defense responses. I will present current research progress in *P. triticina* genomics from collaborative efforts. Nuclear-specific haplotype genome assemblies of over 20 isolates assisted in phasing gDNA and RNA sequences from over 450 isolates to perform an analysis of the mostly clonal North American population structure and delineate genetic lineages. Eleven homeodomain-containing mating-type alleles are identified among the current global genomic resources, six of which are currently found in North America. Assigning these *MAT* allele specificities was greatly revealing and could be correlated in many cases with haplotypes, allowing for the design of mating type-specific multiplex diagnostic assays. These resources were the basis of a pan-genome construction and allele-specific derived effector compendium. Comparative analyses revealed structural differences among haplotype genomes, both among and within clades, including reassortments of mating-type alleles, refining potential origins of genetic lineages. A genetic cross and GWAS analyses have identified potential avirulent effectors while heterologous expression systems have been researched to test their function.

[O31] Over three decades of wheat and barley rust research in Uruguay: a review.

Silvia Germán¹, Venancio Riella², Richard García¹, Gustavo Azzimonti³, Silvina Baráibar⁴, Ruth Scholz⁵, Miguel Angel Raffo⁶, Marina Castro¹, Leonardo Vanzetti¹ and Paula Silva¹

¹Instituto Nacional de Investigación Agropecuaria, Estación Experimental INIA La Estanzuela, Ruta 50, km 11, Colonia, Uruguay, 70006; ²Facultad de Agronomía, Universidad de la República, Garzón 780, Montevideo, Uruguay, 12900; ³Domaine de Bannieres, Castries, France, 34160; ⁴InterGrain Pty Ltd, Perth, Australia, WA 6163; ⁵Instituto Paraguayo de Tecnología Agraria, Ruta VI, km 17, Capitán Miranda, Itapúa, Paraguay, 070501; ⁶Private Consultant, Uruguay 767, Carmelo, Colonia, Uruguay, 70100

Correspondence to: mpsilva@inia.org.uy

This review highlights the status and research on the three wheat rusts and barley leaf rust (BLR) in Uruguay over the past three decades. Until 2017, wheat leaf rust (WLR) was the most prevalent; however, the migration of new wheat yellow rust (WYR) races from Europe led to widespread epidemics that persist today, with local evolution also being demonstrated. Wheat stem rust (WSR) has caused severe but sporadic outbreaks, the last major ones occurring in 2014 and 2015. *Puccinia triticina* exhibits the highest variability, driven by both local evolution and intercontinental migration across the Americas. Breeding for rust resistance has been a priority in INIA's wheat breeding program. Several *Lr* genes conferring all-stage and durable pleiotropic adult plant resistance (APR) are present and/or have been incorporated into INIA's varieties and sources of resistance. The effectiveness of minor *Lr* genes varies across environments, emphasizing the need for strategic gene selection. Two lines resistant in Kenya to WSR were found to carry *Sr28*, along with other locally effective genes. Additionally, *Sr2* coupled with *Fhb1* was successfully introduced into adapted germplasm. Five QTL associated with WYR APR were also identified. For BLR, the *P.*

hordei population has acquired virulence to *Rph3* and *Rph9.z*. European barley germplasm, used directly or through crosses, contributes APR, with *Rph20* conditioning intermediate resistance. Continued surveillance and the introgression of diverse resistance sources will be crucial for maintaining durable rust resistance in Uruguay's wheat and barley varieties.

[O32] Trends in Fusarium Species on Wheat and Tools for Pathogen Identifications in Large-scale Monitoring Programs

Sean Walkowiak¹ and Shimosh Kurera¹

¹Grain Research Laboratory, Canadian Grain Commission, 196 Innovation Drive, Winnipeg, MB, Canada, R3T 2N2

Correspondence to: sean.walkowiak@grainscanada.gc.ca

The fungal genus *Fusarium* contains many diverse species, several of which are plant pathogens. Different *Fusarium* species vary in disease aggressiveness, mycotoxin potential, and environmental adaptations despite causing similar diseases on the same plant hosts. For example, Fusarium Head Blight (FHB) is a disease of wheat that is of significant economic and food safety interest in Canada that can be caused by several species, including *F. acuminatum*, *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. langsethiae*, *F. poae*, *F. pseudograminearum*, *F. sporotrichioides*, *F. tricinctum* and *F. graminearum*. In general, *F. graminearum* is an aggressive pathogen of wheat and it produces trichothecene toxins, whereas *F. avenaceum* is generally not as aggressive and does not have the biosynthetic genes required for trichothecene production. These species can be indistinguishable when inspecting the host plant or contaminated cereal grain; however, the identification of the species is important for understanding pathogen population dynamics, diversity, and risks. We explore the different methods used for *Fusarium* species identification, including traditional culturing and microscopy, DNA barcode sequencing, species-specific DNA tests, whole genome sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) biotyping. We also report on the use of these methods for determining trends in *Fusarium* populations from Fusarium Damaged Kernels of wheat from tens of thousands of samples obtained from across Canada over several decades.

[O33] The largest Canadian population survey and genomic analysis of *Claviceps purpurea* to date

Skyler Shaw^{1,2}, Jim Menzies³, Miao Liu⁴, David Overy⁴, Samia Berraies⁵, Curtis Ponziak⁶, Harmeet Chawla², Sheryl Tittlemier¹, and Sean Walkowiak^{1,2}

¹Canadian Grain Commission, Grain Research Laboratory, Richardson Center for Food Technology Research, 200 Innovation Dr, Winnipeg, MB, Canada, R3T 6B6; ²University of Manitoba, Department of Plant Science, 66 Dafoe Road, Winnipeg, MB, Canada, R3T 2N2; ³Agriculture and Agri-Food Canada, Morden Research and Development Centre, Route 100, Unit 100-101 Morden, Canada, R6M 1Y5; ⁴Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 3701 Carling Ave, Ottawa, ON, Canada, K1A 0C6; ⁵Agriculture and Agri-Food Canada, Swift Current

Research and Development Centre, 1 Airport Rd, Swift Current, SK, Canada, S9H 3X2; ⁶University of Saskatchewan, Crop Development Centre, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8
Correspondence to: shaws347@myumanitoba.ca

Claviceps purpurea (Fr.) Tul. is a fungal pathogen that infects cereal grains and grasses, forming growth of dark- coloured ergot sclerotia in place of normal seed or grain. These sclerotia contain alkaloid mycotoxins that are produced due to expressions of the ergot alkaloid synthesis (EAS) genes. These metabolites have been linked to both serious health detriments and potential medicinal remedies. In this study, we explore thousands of ergot sclerotia from infected cereal grains obtained from the Canadian Grain Commission’s Harvest Sample Program, as well as historical reference samples, to examine the core and dispensable genome of *C. purpurea* on a larger scale than previously performed and construct a pan-genome. Ergot sclerotia were catalogued and selected for culturing based on the province and year of origin. As *Claviceps* are obligate biotrophs that grow slowly in culture, methods were developed and optimized to harvest mycelia for DNA extraction and testing. The generation of long-read genome assemblies using PacBio Hi-Fi whole genome sequencing, and comparative analysis is performed using 70 representative gDNA samples. TaKaRa Next-Gen genotyping will be performed on thousands of sclerotia samples from the 2014-2024 harvest seasons across Canada to explore variation in the EAS gene cluster and additional gene targets potentially associated with disease and population structure. The findings of this project provide further insight into geographic, environmental, and genotypic variance of *C. purpurea*, leading to improved developments in agricultural management of disease and potential health risks.

[O34] Distribution and diversity of abaca bunchy top virus and banana bunchy top virus causing bunchy top of abaca in Caraga, Philippines

Mark Paul Selda Rivarez^{1,2}, Jezmeir Rey Porras¹, Cristal Mae Magadan¹, Arnaldo Gagula³, Rezel Borines¹, Elizabeth Parac¹

¹Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, British Columbia, V6T 1Z4, Canada; ²Department of Agricultural Sciences, College of Agriculture and Agri-industries, Caraga State University, Ampayon, Butuan City, Agusan del Norte 8600, Philippines; ³Department of Geodetic Engineering, College of Engineering and Geosciences, Caraga State University, Ampayon, Butuan City, Agusan del Norte 8600, Philippines.

Correspondence to: paul.rivarez@ubc.ca

The Philippines contributes more than 80% to the total world production of abaca (*Musa textilis*) fiber or ‘Manila hemp’ that is used in various industrial products. However, the growth of abaca industry growth is significantly hampered by yield reduction due to the bunchy top disease (BTD) caused by the single or mixed infection of abaca bunchy top virus (ABTV) and banana bunchy top virus (BBTV) (genus *Babuvirus*, family *Nanoviridae*). Herewith, we surveyed major abaca plantations in Caraga region, Philippines using mapping tools complemented with molecular diagnostics, to generate a distribution map for the incidence of abaca BTD. We also assessed the genetic diversity and phylogenetic relationships of ABTV and BBTV isolates with other global

isolates in order to generate insights on their evolution and geographic origins. We showed that BTD is present in all five Caraga provinces where a total of 395 symptomatic or asymptomatic leaf samples were collected. A representative subset (n=120) were tested for ABTV/BBTV using duplex PCR tests where 84 samples (70%) were positive for BBTV and 66 samples (55%) for ABTV. Interestingly, there is a high rate of ABTV/BBTV co-infection, where 49 samples (41%) tested positive for both viruses. Sequencing of representative isolates and subsequent diversity analyses revealed moderate levels of nucleotide diversity for both viruses with evidence of recombination. Additionally, phylogenetic lineages showed correspondence with the geographic origin of the global isolates. Furthermore, data from PCR tests were used for the MaxEnt (maximum entropy) analyses which provided predictive insights on the possible spread of the disease in the region. Overall, we contributed novel information on the distribution and diversity of ABTV and BBTV. By using innovative predictive analyses, we further contributed to the advancement of the understanding of the epidemiology of the abaca bunchy top disease in a major abaca growing region of the Philippines.

[O35] Overlap between virulence and avirulence function of *Blumeria hordei* AVR_{A13}

Merle Bilstein-Schloemer¹, Wei Shi¹, Sophie Sent¹, Maike Hofmann¹, Isabel ML Saur^{1,2}

¹Institute for Plant Sciences, University of Cologne, 50674 Cologne, Germany; ²The Cluster of Excellence on Plant Sciences (CEPLAS), Germany

Barley MLA immune receptors detect avirulence effectors from multiple fungal pathogens, including rusts and mildews. MLA13 recognises the *Blumeria hordei* (*Bh*) AVR_{A13} effector, but the effector's virulence function remains unknown. Notably, despite being recognized by MLA13, AVR_{A13} is highly conserved within the *Bh* population. In this study, we identified HvSRF3 as the virulence target of AVR_{A13}. Our data suggest that MLA13 evolved to specifically bind the AVR_{A13} residues that are required for its virulence function. We generated transgenic barley to perform proximity-labelling of AVR_{A13} interacting proteins, which identified HvSRF3 as specific interactor of the effector. This receptor-like kinase regulates iron homeostasis in *Arabidopsis* and is highly conserved across dicots and monocots. AVR_{A13} also binds SRF3 in *Arabidopsis*, and AVR_{A13}⁻ overexpression in both barley and *Arabidopsis* increases susceptibility to fungal and bacterial pathogens, which appears to mimic *srf3* knock-out lines. These findings suggest that AVR_{A13} inhibits the SRF3 function, likely through disruption of iron homeostasis. By generating an extensive set of chimeric and mutant AVR_{A13} constructs, we demonstrated that a central AVR_{A13} loop region mediates association with the SRF3 intracellular domain. This same protein loop is crucial for activating MLA13-mediated cell death, as shown in *Nicotiana benthamiana* and barley protoplast-based cell death assays. Together these findings explain why AVR_{A13} is maintained in the global *Bh* population despite being recognized by the MLA13 immune receptor and underlines that *Bh* cannot diversify AVR_{A13} to escape recognition by MLA13 without compromising its ability to modulate SRF3 activity.

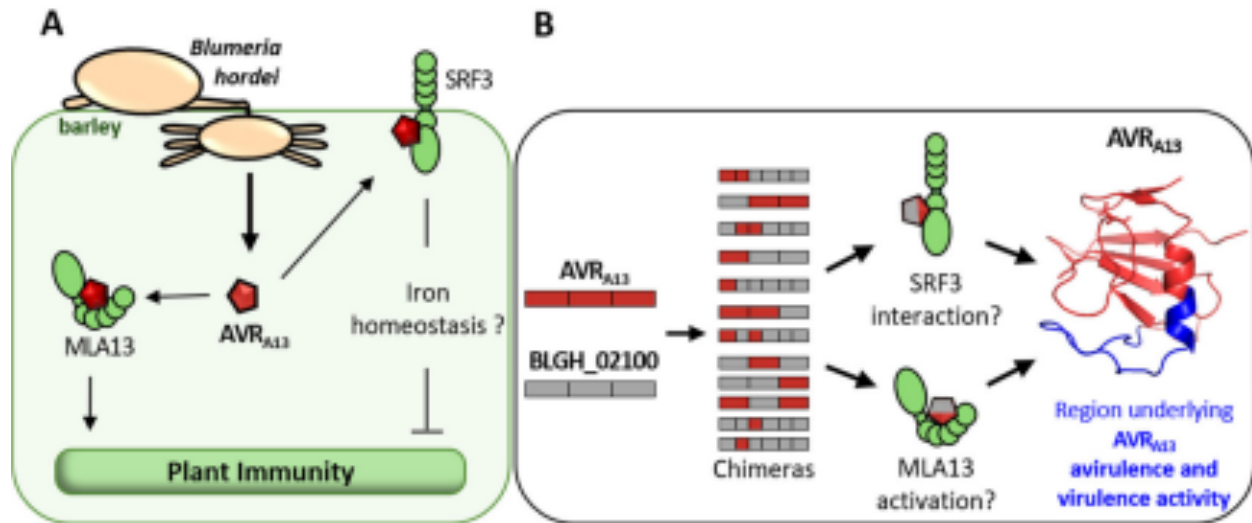


Figure 1 - The *Blumeria hordei* effector AVR_{A13} targets the receptor-like kinase *HvSRF3* in the barley host plant. A) AVR_{A13} is recognized by the host nucleotide-binding leucine-rich repeat receptor (NLR) MLA13, which triggers a plant immune reaction leading to disease resistance. The receptor-like kinase SRF3 is targeted by AVR_{A13} in barley and *Arabidopsis* to suppress plant immunity. AtSRF3 is a key regulator of iron homeostasis, suggesting that AVR_{A13} interferes with iron related processes in the host plant. **B)** Interaction studies and cell death assays with chimeric constructs of AVR_{A13} and the non-functional family member *BLGH_02100* reveal that the same central AVR_{A13} loop region is essential for both interaction with SRF3 and activation of MLA13-mediated cell death.

[O36] The Hidden Genetic Drivers of Leaf Rust Resistance in Wheat: A Focus on Disease Resistance Modifiers

Lamia Aouini¹, Yajun Wang^{1,2}, Emile Cavalet-Giorsa¹, Michael Abrouk¹, Esther Jung³, Beat Keller³, Simon G. Krattinger¹

¹Plant Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia;

²National Key Laboratory of Plant Molecular Genetics, Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China; ³University of Zurich, Department of Plant and Microbial Biology, Zollikerstrasse 107, 8008, Zurich, Switzerland

Correspondence to: lamia.aouini@kaust.edu.sa

Achieving effective disease resistance in wheat remains a major challenge, with genetic background effects playing a significant role in shaping the phenotypic expression of resistance genes. While ‘genetic background effects’ are well documented in the literature, the underlying genetic and molecular mechanisms are not fully understood. Our study focuses on the leaf rust resistance gene *Lr14a*, which encodes an unusual protein composed of twelve N-terminal ankyrin repeats and a C-terminal transmembrane domain. Remarkably, *Lr14a* is strongly influenced by the

genetic background, with certain wheat cultivars being fully susceptible to leaf rust despite carrying *Lr14a*. To investigate this, we used two contrasting, *Lr14a*-containing near-isogenic wheat lines, ArinaLr14a and ArinaLrFor, which exhibited different responses to leaf rust at the seedling stage. While ArinaLrFor displayed the characteristic mesothetic *Lr14a*-type resistance, ArinaLr14a was fully susceptible (Fig. 1, panel A). Segregation analysis of an ‘ArinaLr14a × ArinaLrFor’ mapping population revealed the segregation of a single modifier gene, which was mapped to chromosome arm 1BS (Fig. 1-B; C), spanning a 44 MB genomic region. Further fine-mapping reduced this region to 2 MB (Fig. 1-D), and one top candidate gene has been identified and is currently undergoing functional investigation. Notably, this 1BS region overlapped with the previously reported *Lr75* gene, a minor adult plant resistance gene in ArinaLrFor. Our findings suggest that the *Lr14a* seedling modifier and the *Lr75* adult plant resistance gene may be identical. This study highlights the significance of disease resistance modifiers and lays the groundwork for enhancing leaf rust resistance in the future.

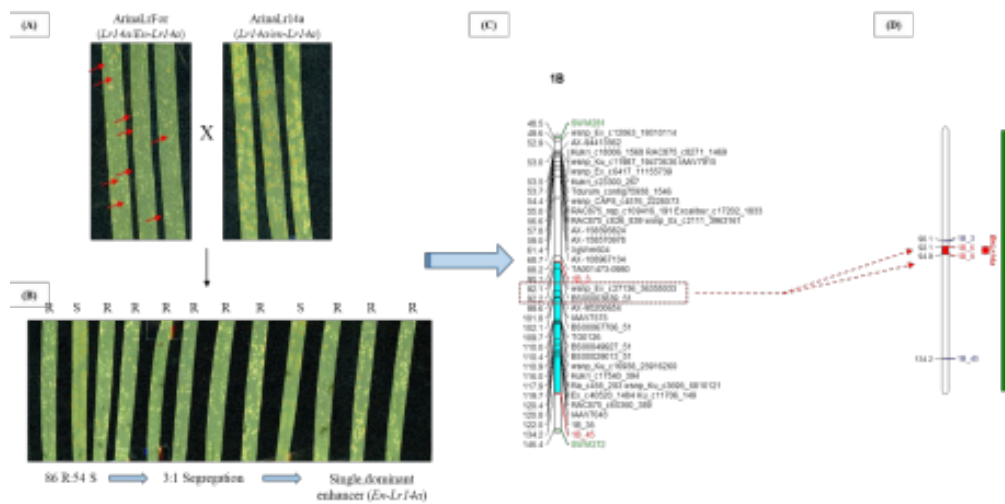


Fig. 1. Mapping of the *En-Lr14a* modifier gene. (A) Two near isogenic bread wheat lines, ArinaLrFor and ArinaLr14a, both carrying the leaf rust resistance gene *Lr14a*. While ArinaLr14a is susceptible to leaf rust at seedling stage, ArinaLrFor shows a mesothetic resistance response, with both fully developed leaf rust uredia and hypersensitive flecks (red arrows) occurring on the same leaf. This suggests the presence of an enhancer, *En-Lr14a*. (B) A single, dominant *En-Lr14a* segregated on the derived ‘ArinaLr14a × ArinaLrFor’ mapping population. (C) The *En-Lr14a* was mapped to chromosome arm 1BS, spanning a 44 MB genomic region. (D) Subsequent fine-mapping reduced this region to 2 MB. This region is overlapping with the previously identified minor adult leaf rust resistance gene *Lr75*. Our findings suggest that the *En-Lr14a* and the *Lr75* may be identical.

[O37] Metagenomics-assisted near chromosome level assembly and annotation of *Puccinia graminis* f. sp. *tritici* isolate UK01

Karthick Gajendiran¹, Angel Angelov², Patrick Driguez², Alexander Putra², Nagarajan Kathiresan², Brande B.H. Wulff¹

¹Plant Science Program, Biological and Environmental Science and Engineering Division (BESE), King Abdullah University of Science and Technology (KAUST); Thuwal, Saudi Arabia; ²Bioscience Core Lab, King Abdullah University of Science and Technology (KAUST); Thuwal, Saudi Arabia
Correspondence to: brande.wulff@kaust.edu.sa

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) poses a risk to wheat production globally. In this study, we present a near-complete genome assembly and high-quality annotation of the English isolate UK01, a representative of the "Digalu" *Pgt* tribe that caused an epidemic in Ethiopia in 2014. To sequence and assemble the UK-01 genome, we infected *Aegilops tauschii*, a diploid wheat progenitor, and isolated high molecular weight (HMW) DNA from heavily infected tissue. This bypassed the difficulty of extracting HMW DNA from fungal spores. PacBio-sequencing of the metagenomic sample, followed by genome assembly and HiC scaffolding, allowed us to effectively resolve the dikaryotic haplotypes of *Pgt*. RNA-Seq and IsoSeq data was used to perform genome annotation utilizing the BRAKER3 and Funannotate pipelines, which revealed accurate gene models and identified 2444 candidate effector genes. This resource will be useful for improving our understanding of stem rust effector biology, develop molecular surveillance tools, and inform resistance breeding programs.

[O38] Pattern Recognition Receptors (PRRs) Regulate Resistance to Fusarium Head Blight

Maryam Nourimand¹, Rajagopal Subramaniam¹

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada, K1A0C6

Correspondence to: Rajagopal.subramaniam@agr.gc.ca

Our previous study demonstrated that a membrane receptor APEX and the danger-associated molecular patterns (DAMP) receptor RLK7 act as negative regulators of immunity against Fusarium head blight (FHB), an economically important disease of cereals. In the present study, we have further elucidated the function of the two receptors using Arabidopsis. The double mutant *rllk7/apex* that display enhanced resistance to Fusarium can be restored to disease phenotype with overexpression of APEX from wheat. However, overexpression of wheat RLK further enhanced resistance in the *rllk7/apex* mutant background. To validate the role of APEX as the negative regulator, an RNA-seq was performed with *rllk7/apex* and was compared it to both wildtype and *rllk7/apex* complemented with *TaAPEX*. The analyses identified previously uncharacterized genes involved in FHB resistance that will serve the wheat breeding community with novel markers and potential targets for gene editing.

[O39] Gene Transformation with Syntaxin of plants, Lectin Receptor Kinase or executor1 provides Resistance to the Necrotroph Pathogen, *Sclerotinia sclerotiorum*

Lone Buchwaldt, Edis Dzananovic, Fuyou Fu, Alan Davies, Jonathan Durkin, Helen Lui.

Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada

Correspondence to: Lone.Buchwaldt@agr.gc.ca

Sclerotinia sclerotiorum is a necrotrophic pathogen on dicotyledonous plant species. Ascospores form infection- cushions on dead organic material, and synthesise secondary metabolites killing cells in advance of hyphae. Our objective was to enhance stem resistance in canola to protect seed yield. Defense genes were identified in a genome wide association study using a world collection of *B. napus* lines. Genes were cloned from a resistant line and transformed into a susceptible line, DH12075. Transformants with a single homozygous gene-insert were identified in T₃-T₆. Gene expression studies confirmed each inserted gene was expressed constitutively by the CaMV35S promotor. Resistance was identified by inoculation of stems with mycelium plugs resembling infection- cushions. The *EXECUTER1* gene was large with 11 exons. The EX1 protein is located in chloroplasts, activated by singlet oxygen (O⁻¹) from excessive light resulting in programmed cell death. We showed the gene was also triggered by *Sclerotinia* resulting in 0-17% collapsed stems compare to 61% in DH12075 (Table 1). Lines transformed with one of four gene copies of *SYNTAXIN OF PLANTS 121* showed 0-33% collapsed stems. The protein is located in the plasma membrane and facilitates fusion with vesicles, followed by secretion of contents into the apoplast. *LECTIN RECEPTOR KINASE A4.3* code a protein embedded in the plasma membrane comprising a lectin domain that senses microorganisms, and a kinase domain that trigger defense pathways. Lesions on resistant stems were confined to the outer epidermis and mesophyll and rarely invaded the vascular tissue. The quantitative resistance was evident all through to plant maturity.

Cloned and inserted gene	Gene function	Location of cloned gene on <i>B. napus</i> chromosome	Number of exons	Exon size, nt	Constitutively expressed	Sclerotinia reaction: Collapsed stems
<i>EXECUTER1</i>	Programmed cell death	A8	11	2544	Yes	0-17%
<i>LecRK A4.3</i>	Recognition and defense signaling	C3	2	1947	Yes	17-33%
	Facilitates fusion of the plasma membrane with vesicles, and	A3	2	1064	Yes	0-33%
<i>SYNTAXIN OF PLANTS 121</i>	secretion of contents into the apoplast	A5	2	1020	Yes	0-33%
		C3	2	1903	Yes	0-33%
		C5	2	1553	Yes	0-33%
DH12075	Untransformed		na	na	na	61%

[O40] The dual role of *Olpidium brassicae* in clubroot disease development in canola

Jacqueline Gonzalez-Sacoto¹, Jennifer Town¹, and Bobbi L. Helgason¹

¹Department of Soil Science, University of Saskatchewan, 51 Campus Dr, Saskatoon, SK S7N 5A8;

²Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, 107 Science Pl, Saskatoon, SK S7N 5E2

Correspondence to: kzx826@usask.ca

The root-associated microbiome plays a key role in plant health and disease progression. While *Olpidium brassicae* is frequently associated with canola roots and rhizosphere, its role in disease development remains poorly understood. This study explores the interaction between the most

prevalent strains of *O. brassicae* (d3f1 and f509) in Western Canada and *Plasmodiophora brassicae* pathotype 3H, the pathogen responsible for clubroot disease. Using soils from Saskatoon, SK, and Lacombe, AB, growth chamber experiments compared the root-associated microbiomes of clubroot-susceptible Westar and clubroot-resistant InVigor L241C canola cultivars. Inoculations of *O. brassicae* (d3f1/f509) and *P. brassicae* 3H were performed individually and in combination. Disease severity was assessed at 35 days post-inoculation (dpi). DNA was extracted from roots and rhizosphere samples (7 dpi and 35 dpi) and quantitative PCR (qPCR) and droplet digital PCR (ddPCR) were used to quantify *O. brassicae* and *P. brassicae* abundances. Additionally, the root-associated microbiome was sequenced using the MiSeq V2 kit. Preliminary findings reveal contrasting effects: in Lacombe soil, the co-inoculation of *O. brassicae* and *P. brassicae* exacerbated clubroot symptoms, whereas in Saskatoon soil, it prevented symptom development. The strains d3f1 and f509 consistently dominated plant roots, suggesting potential niche competition with *P. brassicae*, but there was no apparent competition between the strains. Composition and diversity of root-associated bacteria and fungi will help to understand how interactions between *O. brassicae* and *P. brassicae* influence clubroot development and the canola root-associated microbiome in these two soils. This work provides critical insights into leveraging microbial interactions for Clubroot management in canola.

[O41] Characterizing the ClToxB protein of *Colletotrichum lentis* through gene knockout via protoplast-mediated transformation

Caitlyn Kolb¹, and Sabine Banniza²

¹Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8; ²Crop Development Centre, University of Saskatchewan, College of Agriculture and Bioresources, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8

Correspondence to: caitlyn.kolb@usask.ca

Anthracnose, caused by the fungal pathogen *Colletotrichum lentis* Damm, has developed into one of the most damaging diseases of cultivated lentil (*Lens culinaris* Medik.) in western Canada. *Lens ervoides* Grande is a wild relative of the cultivated *L. culinaris* that has been used as a source of resistance to this pathogen. *Colletotrichum lentis* secretes toxic effector proteins that manipulate host cell physiology and promotes infection. ClToxB is a host-specific effector secreted by *C. lentis* that likely induces cell death during the pathogen's switch from biotrophy to necrotrophy. To test this hypothesis, *ClToxB* was knocked out in the genome of a *C. lentis* isolate. Protoplast-mediated transformation was used to introduce a gene fragment into the pathogen that replaced the *ClToxB* with antibiotic resistant *BAR* via homologous recombination. The mutant pathogen, alongside the wild type isolate, will be inoculated onto susceptible and resistant *L. ervoides* lines, and *L. culinaris* plants. Plants will be monitored post-inoculation to compare disease symptoms in response to infection by the mutant and wild type isolates.

[O42] Current Status of Wheat Disease Surveillance and Early Warning

Dave Hodson

Director, Wheat Disease Early Warning Advisory System (DEWAS); Principal Scientist, CIMMYT

Correspondence to: D.Hodson@cgiar.org

An overview of the current status of wheat disease surveillance and early warning will be outlined. Key highlights of work in East Africa and South Asia will be the focus. The current operational early warning system for globally important wheat diseases (wheat rusts, wheat blast, FHB) will be described. The success of the system in detecting emerging new threats to wheat production will be outlined, along with new advances in diagnostics, forecasting and risk assessment. Current knowledge on the emergence and interchange of pathogens between different geographical regions will be highlighted.

[O43] Forecasting Wheat Diseases in Africa and South Asia as Part of the Wheat Disease Early Warning and Advisory System

Jacob Smith

Postdoctoral Research Associate, Epidemiology & Modelling Group, University of Cambridge

Wheat diseases pose recurring threats to food security due to factors including climate change, land use change, and emerging virulent strains. Rapid response to a wheat disease outbreak requires a timely and accurate estimate of disease risk. Building on a century of literature, over a decade of research, and years of development in pilot studies, a Wheat Disease Early Warning and Advisory System (DEWAS) is now in operation for eight countries in Africa and South Asia. One component of the interconnected and collaborative DEWAS project is the risk forecasting of wheat diseases, including the wheat rusts and wheat blast. At the core of the forecasting system are components for meteorologically driven aerobiological spore release and dispersal from known sites of infection, environmental suitability conditions for infection for stem, stripe and leaf rust and an integrated epidemiological model for stem rust. Near real-time ground surveys are uploaded automatically by trained personnel as input to initiate the models and accessed via the Wheat Rust Toolbox. State-of-the-art seven-day weather forecasts for target countries are provided by the UK Met Office, from which daily forecasts of wheat rust risks are calculated. Forecasting is also expanding to include wheat blast in Africa. Key to making the system sustainable and adaptable are capacity building through training and modular software development. Evaluation shows that integrating near real-time surveys and modelling long-distance spore dispersal improves prediction accuracy. The flexible framework can also be applied to other airborne plant diseases.

[O44] Wheat Diseases Research Status in Ethiopia

Jemal Tola Horo

Director, Ambo Agricultural Research Center; and Researcher, Plant Pathology

Correspondence to: tolajemal@gmail.com

Ethiopia, the largest wheat producer in Sub-Saharan Africa, encounters considerable challenges from wheat diseases that threaten food security and agricultural sustainability. Wheat serves as a critical staple crop in Ethiopia, playing a significant role in food security and the national economy. However, its production is often affected by several diseases, such as rusts, fusarium head blight, and septoria. Research efforts in Ethiopia have made notable progress in understanding, managing, and mitigating these challenges. Key advancements encompass the release of over 115 bread wheat and 47 durum wheat varieties via breeding programs, with a particular focus on managing rust diseases, which are the most destructive. Collaborative initiatives among Ethiopian research institutions and international organizations, including CIMMYT and ICARDA, have accelerated the advancement of high-yielding, disease-resistant cultivars. Furthermore, research on disease epidemiology has enhanced the Wheat Rust Early Warning System (EWS), enabling timely interventions. The system has been providing an automatic seven-day forecast risk map along with weekly advisories and alerts. Integrated disease management strategies that incorporate resistant varieties, cultural practices, and fungicide applications have been promoted to enhance sustainable wheat production. Despite these achievements, challenges persist, including the emergence of new pathogen strains (stem rust and yellow rust races), re-emerging diseases such as fusarium head blight, limited access to improved seeds for smallholder farmers, and weakness in extension services. Ongoing research emphasizes the need for continuous surveillance, developing multi-disease resistant wheat varieties, farmer education, and investment in modern technologies to ensure long-term resilience against wheat diseases in Ethiopia.

[O45] First Detection and Genetic Characterization of *Magnaporthe oryzae* pathotype *Triticum* in Uruguay

Paula Silva

Associate Scientist, National Institute of Agricultural Research (INIA Uruguay)

Correspondence to: mpsilva@inia.org.uy

Wheat blast, caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), is an emerging and highly destructive disease in global wheat production. Previously confined to South America—especially Brazil, Paraguay, and Bolivia—MoT had not been reported in Uruguay until the 2023 season. That year, unusually favorable weather conditions in northwestern Uruguay, similar to those associated with outbreaks in Brazil, triggered intensive disease surveillance. Seventeen wheat spike samples showing blast-like symptoms were collected from 11 fields; nine tested positive for *M. oryzae* based on morphological traits. Four monosporic isolates were recovered, and three underwent molecular and genomic analyses. PCR confirmed their identity as MoT, and pathogenicity assays showed that all could infect both leaves and spikes of susceptible wheat genotypes. Whole-genome sequencing and phylogenetic analysis of 57 *M. oryzae* strains—spanning *Triticum*, *Lolium*, and *Eleusine* hosts—revealed that the Uruguayan strains are closely related to some Brazilian MoT isolates but genetically distinct from the B71 lineage implicated in Asia and Africa. Notably, isolates PyrUy10.1 and PyrUy14.1 shared 99.9% of SNPs, while PyrUy11.1 showed only 73%

similarity, suggesting multiple introductions and the coexistence of genetically distinct lineages. These findings confirm the first detection of MoT in Uruguay and underscore the risk of cross-border pathogen movement. The presence of diverse MoT lineages presents new challenges for disease management and highlights the urgent need for ongoing monitoring in areas with favorable conditions for wheat blast development.

[O46] Dissecting Tan Spot Resistance in Mediterranean Durum Wheat

Marwa Laribi^{1,2}, Sarrah Ben M'Barek^{2,3,4}, Sandra M. Velasco-Cuervo¹, Sheau-Fang Hwang², Amor H. Yahyaoui^{2,5} and Stephen E. Strelkov²

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada; ²CRP Wheat Septoria Precision Phenotyping Platform, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia; ³Regional Field Crops Research Center of Beja (CRRGC) BP 350, 9000 Beja, Tunisia; ⁴Laboratory of Support for the Sustainability of Agricultural Production Systems in the Northwest Region, Higher School of Agriculture of Kef (ESAK), University of Jendouba, Le Kef, Tunisia; ⁵Borlaug Training Foundation, Fort Collins, CO 80523 USA

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is a major constraint to durum wheat production. This study examined the genetic diversity and resistance to tan spot in 258 durum wheat accessions from eleven Mediterranean countries. All accessions were evaluated at the adult stage under field conditions over two seasons and at the seedling stage under controlled conditions for resistance to Ptr races 2, 3 and 5. Genetic analysis was conducted using 8,015 single nucleotide polymorphism (SNP) markers and 28,200 SilicoDArT markers. The results highlighted extensive genetic exchange among Mediterranean durum wheat populations and supported two primary dispersal pathways: a northern route (encompassing the northern and eastern Mediterranean basin) and a southern route (including North Africa and the Iberian Peninsula). Both race-specific and race-non-specific resistance were identified, with several genotypes exhibiting resistance at both the adult and seedling stages. Genome-wide association study (GWAS) identified several SNPs associated with tan spot resistance, explaining up to 24.2% of the phenotypic variation and mapping across all chromosomes. The accessions were also screened for the presence of *Tsn1*, a gene conferring sensitivity to the fungal necrotrophic effector ToxA. Interestingly, the presence of *Tsn1* did not consistently result in susceptibility to ToxA-producing isolates, suggesting that the ToxA-*Tsn1* interaction may not play a significant role in tetraploid wheat and that additional factors influence the Ptr-durum wheat interaction. These findings underscore the complexity of tan spot resistance in durum wheat and provide a comprehensive overview of available genetic resources for breeding programs.

[O47] Evaluation of wheat for resistance to bacterial leaf streak

Valentina Anastasini¹, Lipu Wang¹, Constanza Fleitas, Kelly Turkington², and Randy Kutcher¹

¹Cereal and Flax Pathology, Department of Plant Sciences, University of Saskatchewan, College of Agriculture and Bioresources, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8; ²Lacombe Research and Development Centre, Agriculture and Agri-Food Canada, 6000 C and E Trail, Lacombe, Alberta, Canada.

Correspondence to: randy.kutcher@usask.ca

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens*, is a foliar disease favored by warm, humid conditions. It has become increasingly problematic in North America. This transition from a sporadic to a widespread issue requires effective disease management strategies. An integrated pest management (IPM) approach is essential, including cultural, biological, and chemical practices to minimize risks. However, given the lack of effective cultural and chemical options against BLS, the development of resistant cultivars is a major IPM strategy. This project evaluated a diverse panel of 200 elite wheat lines and cultivars under both field and controlled conditions to identify potentially resistant commercial cultivars and promising sources of resistance in elite breeding material. Field trials were conducted across multiple site-years, and growth chamber experiments were performed to assess resistance under controlled conditions. Preliminary results indicate significant variation in resistance and susceptibility among current Canadian wheat cultivars. There was no correlation between field and growth chamber results, highlighting the complexity of BLS resistance. The best-performing material exhibited high levels of resistance, with disease severity scores as low as 3.3 on a 0-9 scale, while the most susceptible lines had scores up to 7.5. Promising elite lines from the University of Saskatchewan breeding programs were identified, providing valuable resources for future breeding efforts. These findings enhance our understanding of BLS resistance and support the development of resistant wheat cultivars.

[O48] Screening for (and trying to understand) resistance and loss-of-susceptibility to powdery mildew in *Cannabis sativa*

Youssef Moutahir¹, and [David L. Joly](mailto:David.L.Joly@umoncton.ca)¹

¹Cannabis Innovation and Research Centre, Université de Moncton, 18 avenue Antonine-Maillet, Moncton, NB, Canada, E1A 3E9

Correspondence to: david.joly@umoncton.ca

Cannabis powdery mildew, primarily caused by *Golovinomyces ambrosiae*, poses a significant threat to *Cannabis sativa*, impacting yield, quality, and profitability. We recently initiated a sustained effort to identify sources of resistance to powdery mildew by screening a diverse collection of over 150 cannabis cultivars under controlled inoculation conditions. We evaluated resistance-associated phenotypes across cultivars, and highly resistant individuals were selected for molecular analysis, focusing on known susceptibility genes such as *CsMLO1*, to differentiate between resistance and loss-of-susceptibility mechanisms. Preliminary results identified several promising cultivars, including a subset for which resistance correlated with the known *CsMLO1* gene inactivation. However, *CsMLO1* inactivation did not consistently result in resistance, highlighting variability in resistance/susceptibility mechanisms across genetic backgrounds. Among resistant cultivars not harbouring the *CsMLO1* inactivation, a completely immune cultivar was identified, and crossed with a susceptible cultivar. Genetic analyses suggested two dominant genes, one conferring partial resistance and one conferring complete resistance. Two other cultivars exhibited strong resistance during vegetative growth that appeared to be lost after

transitioning into flowering. These cultivars provide valuable candidates to increase our understanding of this pathosystem, as well as for breeding programs aimed at enhancing powdery mildew resistance in cannabis. This research will contribute to sustainable cannabis cultivation by facilitating the selection of disease-resistant lines, reducing reliance on the limited control options available to cultivators.

[O49] Decoding knockout resistance: Insights into a AAC Tenacious TILLING population

Maria Antonia Henriquez¹, Philip Walker¹, Curtis J Pozniak², Valentyna Klymiuk², Sean Walkowiak³, Kirby T Nilsen⁴, Srinivas Sura¹, Santosh Kumar⁴

¹Morden Research and Development Centre, Agriculture and Agri-Food Canada, Unit 101 Route 100, Morden, MB, R6M 1Y5, Canada; ²Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada; ³Grain Research Laboratory, Canadian Grain Commission, 196 Innovation Drive, Winnipeg, MB, R3T 6C5, Canada; ⁴Brandon Research and Development Centre, Agriculture and Agri-Food Canada, 2701 Grand Valley Road, Brandon, MB, R7A 5Y3, Canada

Correspondence to: mariaantonia.henriquez@agr.gc.ca

AAC Tenacious remains the only registered spring wheat cultivar in Canada rated as resistant to Fusarium head blight (FHB). While AAC Tenacious has superior FHB resistance compared to other cultivars, its agronomic performance is less competitive. We employed gamma irradiation mutagenesis to generate a Targeting Induced Local Lesions In Genomes (TILLING) population of 1046 AAC Tenacious mutant lines. Detailed phenotypic characterization under field conditions identified mutant lines that exhibited variation in agronomic and pest resistance traits, including noticeable range in semi-dwarf stature (40-90 cm). We have compiled top performing mutants based on their FHB severity and consistency in susceptibility across M3 field and M4 Type II resistance data. A total of 100 lines have been selected for whole genome sequencing using the Illumina NovaSeq platform. Of these 82 lines showed strong and consistent FHB susceptibility, while 12 mutant lines expressed FHB resistance combined with reduced height. To facilitate deeper analysis, we generated a reference-quality genome assembly of AAC Tenacious using the latest long-read sequencing and scaffolding technologies. Comparative analysis between the AAC Tenacious assembly and short-read sequences of FHB-susceptible TILLING mutants will enable the identification of specific genes and loci directly associated with FHB-resistance in AAC Tenacious. The germplasm, genotypic and phenotypic data generated from this project will benefit Canadian wheat breeding and research programs. These findings will have a positive impact on the development of future wheat cultivars in Canada by improving yield and quality while reducing our dependence on fungicides for the FHB control.

[O50] Identifying and Genetic Mapping of Resistance Genes in Wheat Against Bacterial Leaf Streak

Malini Jayawardana¹, Silvia Rosa², Dilantha Fernando¹

¹Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 2 740, Chemin Trudeau, Saint-Mathieu-de-Beloeil, QC J3G 0E2

Correspondence to: dilantha.fernando@umanitoba.ca

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* (Xt) poses a threat to wheat and barley worldwide. Although BLS is reported as a devastating disease in several countries, it was not an issue in Canada previously. BLS was recently identified as an emerging disease in Canadian wheat-growing regions, including Alberta, Saskatchewan, and Manitoba. Therefore, studying BLS and identifying potential management strategies to control BLS in Canada is essential. Among BLS management strategies, host resistance plays an important role. Currently, knowledge of host resistance in Canadian germplasm is lacking. To map the resistance genes in wheat against BLS, we have used a double haploid (DH) wheat population, which was identified previously, showing segregation for BLS under the field conditions and was identified to have quantitative trait loci (QTLs) associated with leaf and stripe rust. The DH population was previously genotyped by Rosa et al. 2019 (Genetics and Resistance, 109: 1760- 1768). The population was phenotyped by inoculating with BLS pathogen *Xanthomonas translucens* pv. *undulosa* (Xtu) strains through syringe infiltration. The phenotyping and genotyping data from the DH population were used to identify QTLs with QGene software (version 4.4). The QTL analysis found two QTLs residing on 4A and 7D chromosomes. The stability of the QTLs will be further analyzed by phenotyping with multiple Xtu isolates and under different environmental conditions. Identifying QTLs associated with BLS in this DH population will be important in the future when developing cultivars resistant to multiple diseases.

[O51] Enhancing our ability to rapidly detect and respond to wheat rust and blast outbreak

Diane Saunders

Head of the Crop Genetics Department, John Innes Centre

Correspondence to: diane.saunders@jic.ac.uk

Wheat production is under constant threat from rapidly evolving, transboundary fungal diseases, with rust and blast diseases among the biggest concerns. Wheat rusts have ravaged wheat cultivation ever since the dawn of agriculture about 10,000 years ago, with new variants continually emerging and spreading rapidly on a global scale. In contrast, wheat blast is a recently emergent disease that can cause complete crop loss under favourable conditions, although, it remains more limited in geographical distribution. In both cases rapid and accurate diagnostics is essential to inform effective disease management decisions. To address this, within the DEWAS programme we have developed and deployed advanced genomic-based diagnostic methods across South Asia, South-Central and East Africa. This includes further development and deployment of the Mobile And Real-time PLant disEase (MARPLE) diagnostics platform, that has reduced the speed of strain-level diagnostics for yellow and stem rust to just 48 hours. This new rapid diagnostic system is now situated in multiple countries across East Africa and South Asia to keep track of the changing landscape of wheat rust populations. We also further developed and trialled a new point-of-care real-time diagnostic method for wheat blast to provide accurate and timely diagnostics in South Asia and South-Central Africa. This is further complemented by detailed genetic analysis using the T-Plex genotyping platform that provides genomic-based insight into the distribution of specific

lineages of the wheat blast pathogen worldwide. Together, these advances are helping rapidly detect changes in rust and blast populations to ensure timely deployment of intervention strategies.

[O52] Monitoring of Wheat rust diseases and its Significance in Disease Management in Nepal

Suraj Baidya¹*, Roshan Basnet², Basistha Acharya¹, Laxman Aryal², Mahesh Subedi³, Dhruva Bahadur Thapa³, and David Hodson⁴

¹National Plant Pathology Research Centre, NARC, Khumaltar, Nepal; ²National Wheat Research Program, NARC, Rupendehi, Nepal; ³National Plant Breeding and Genetic Research Centre, Khumaltar, Nepal; ⁴CIMMYT-Nepal, Khumaltar, Lalitpur, Nepal

Correspondence to: *Suraj_baidya222@yahoo.co.in

Wheat is one of the major staple crops in Nepal, providing food security and income for millions of farmers. However, wheat production is significantly affected by various diseases such as rusts, blight, mildews. Effective disease management is crucial to maintain yield and crops quality. One of the key components of disease management is disease monitoring, which involves regular observation and early detection and disease alert system. Every year, systematic surveillance has been conducted in the major wheat production areas of Nepal utilizing the ODK app. The information showed that the Himalayan regions of Nepal serves as a primary hotspot and potential origin site of certain wheat pathogens. In recent years, the emergence of a new variant (TTKTT) of UG99 pathotype and TTKTF and TKFTF of Clade IV-F genetic group of the stem rust pathogen, along with increasing genetic diversity among yellow rust (Pst6, Pst16) and leaf rust (121R60-1, 121R63-1, 93R15, 21R63, 121R55-1) populations, underscores intensified surveillance and research attention. Similarly, given the consistently increasing trend of powdery mildew and leaf blight severity on released Nepalese genotypes in recent years, it is essential to develop wheat genotypes with broad-spectrum resistance to multiple diseases and diverse pathogen populations, particularly in context of Nepal.

[O53] Battling Wheat Rust in Kenya: The Rise of PstS16 Race and GWAS-Based Insights into Rust Resistance in CIMMYT's Elite Germplasm

Naeela Qureshi¹, and Sridhar Bhavani²

¹International Maize and Wheat Improvement Center (CIMMYT), Carretera Mexico-Veracruz Km. 45, EL-Batan, Texcoco 56237, Mexico; ²International Maize and Wheat Improvement Center (CIMMYT), ICRF Campus, United Nations Avenue, Gigiri, Nairobi P.O. Box 1041-00621, Kenya

Correspondence to: n.qureshi@cgiar.org

Stripe rust (*Puccinia striiformis* f. sp. *Tritici*; YR) and stem rust (*Puccinia graminis* f. sp. *Tritici*; SR) remain major threats to global wheat production due to evolving virulences and emerging races. This study reports the spread of the PstS16 stripe rust race (virulence: 1,2,3,(4),-,6,7,8,9,-,-,17,-,-,25,27,32,-,AvS,-) in Kenya, breaking resistance in previously resistant varieties. Race-typing and monitoring across major wheat-growing regions confirmed its spread, emphasizing the need for improved breeding strategies. To address this challenge, a genome-wide

association study (GWAS) using a linear mixed model was conducted on 829 elite parental wheat lines from CIMMYT's breeding program. The panel was field-phenotyped in Njoro, Kenya, over two years, where stripe rust data showed a 70% correlation, and stem rust showed a 60% correlation, indicating consistent disease responses. Genotyping with the 40K XT SNP array identified significant markers for stem rust resistance on chromosomes 1A.1 (88 Mb), 1A.2 (585 Mb), 1B (650 Mb), 1D.1 (10-12 Mb), 1D.2 (410-418 Mb), 2A (3-6 Mb), 2B (531 Mb), 2D (2.5 Mb), 3A (22-25 Mb), 3B (690 Mb), 3D (165 Mb), 4A.1 (2 Mb), 4A.2 (690-700 Mb), 4B.1 (66 Mb), 4B.2 (620 Mb), 5A (687 Mb), 5B (538 Mb), 6A.1 (2 Mb), 6A.2 (615 Mb), 6B (618-620 Mb), 7A (697 Mb), 7B (621 Mb) and 7D (330 Mb) with several regions overlapping known SR genes (*Sr8*, *Sr9h*, *Sr13*, *Sr22*, *Sr25*, *Sr38*, *Sr50*, *Sr58*). For YR resistance, significant SNPs were identified on chromosomes 1A (580-592 Mb), 1B (682 Mb), 2A (744 Mb), 2B (26.8 Mb), 3B (23 Mb), 4B (670-672 Mb), 6A (7 Mb), 6B (98 Mb), 6D (8.6 Mb), and 7B (99 Mb) aligning with some of the known resistance genes; *Yr29*, *Yr78*, and *Yr81*. Candidate genes were identified within most QTL regions, providing valuable targets for marker-assisted selection. Further studies are underway to characterize these genomic regions.

[O54] Harnessing the Power of Free and Open-Source Software for Disease Surveillance

Yoseph Alemayehu

Research Officer, CIMMYT

Of the many constraints of wheat production, pests and diseases are important biotic constraints. Pest and diseases surveillance is one of the key activities carried out by National Plant Protection Organizations (NPPO's). It helps to them gain knowledge of the prevalence of pests and diseases. Despite on-going efforts, however, gathering timely and quality-controlled data has been a challenge for many NPPO's in developing countries. Wilkinson et.al. (2016) argues for an urgent need of improving the infrastructure to support the reuse of scholarly data. They coined an acronym called FAIR data. FAIR data is data that meets the FAIR principles. These are Fundability, Accessibility, Interoperability and Reusability. Nonetheless, abiding to FAIR principles is not only limited by willingness to share data but the investment required to ensure acquisition and storage of quality data in a sustainable platform. This calls for affordable platforms that can be used by NPPO's. This paper reviews surveillance systems implemented by different organizations. It also discusses the power of Free and Open-source (FOSS) tools that are available but largely overlooked. FOSS is an application that is available for free, and its source code is available for review or further development. The paper also shares experience from the key wheat diseases surveillance activity in the Disease Early Warning and Advisory System (DEWAS) project that employed the Open Data Kit (ODK). The use of the ODK helped the collection of near-real time data to ensure timely information and made sample tracking possible.

[O55] Breakdown of Yr15 Resistance in the UK

Charlotte Nellist

Pathology Programme Lead, Niab

Correspondence to: charlotte.nellist@niab.com

The United Kingdom Cereal Pathogen Virulence Survey (UKCPVS) was established in 1967 following a major yellow rust outbreak on the previously resistant wheat variety Rothwell Perdix. Since then, *Puccinia striiformis* f. sp. *tritici* (*Pst*), the causal agent of yellow rust, has been routinely monitored across the UK wheat growing regions.

In spring 2025, unusually high levels of yellow rust were observed by the Niab Northern trials team on wheat varieties rated as highly resistant at both the seedling and the adult plant stages. Similar reports and samples were received from across the industry. Pathotyping of three of these early-season isolates on a wheat differential line set confirmed that all three carried virulence to *Yr15* – a major, broad-spectrum, all-stage resistance gene first identified in the 1980s and widely deployed worldwide. Genotyping using KASP markers revealed that *Yr15* is present in many UK AHDB Recommended List varieties previously classified as resistant at the young plant stage. Collectively these account for at least 58.7% of the UK certified seed market, excluding farm-saved seed. MARPLE diagnostics genotyping performed on two of the early isolates (from varieties Champion and KWS Dawsum) indicate that the new virulent pathotype likely represent a locally evolved variant of the European *PstS10* (Warrior -) lineage, rather than a recent exotic incursion. The widespread cultivation of *Yr15*-carrying varieties has likely exerted strong selection pressure on the pathogen population, facilitating the emergence of virulence in the current season. Ongoing work includes transcriptome and whole genome sequencing of these isolates to investigate the underlying genetic changes associated with the breakdown of resistance.

[O56] Genetic architecture of host species specificity in grasses to fungal pathogens

Matthew J. Moscou^{1,2}

¹USDA-ARS Cereal Disease Laboratory, 1551 Lindig Street, Saint Paul, MN, 55108, USA;

²Department of Plant Pathology, University of Minnesota, Saint Paul, MN, 55108, USA

Correspondence to: matthew.moscou@usda.gov

In 1894, Jakob Eriksson observed that cereal rusts (*Puccinia* spp.) exhibited a preference for the grass host species from which they were isolated. This observation established the concept of host-species specificity. These specialised forms, designated *formae speciales*, have been observed in several plant-filamentous pathogen systems including: powdery mildews of cereals, *Fusarium* vascular wilt, blights of grasses, downy mildews of Asteraceae, white rusts of Brassicaceae, and smuts of cereals. To understand the genetic architecture underlying the inappropriate host status of barley to wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*; *Pst*), we screened a large diverse collection of barley germplasm including elite, landrace, and wild barley (*H. vulgare* subsp. *spontaneum*). We found that susceptibility to *Pst* was rare (<1%; N=196) in domesticated barley, whereas common in wild barley (~25%; N=313). Using a collection of >30

populations (F₂, RIL, BC₁, and DH) derived from a panel of 25 diverse (elite to wild) resistant barley accessions, we mapped resistance and identified four major *R* genes: *Rps6*, *Rps7*, *Rps8*, and *Rps10*. *Rps6*, *Rps7*, and *Rps10*-mediated resistance is conferred by nucleotide binding, leucine-rich repeat (NLR) proteins, whereas *Rps8*-mediated resistance is conferred by a genetic module: a leucine-rich repeat receptor kinase LRR-RK and Exo70FX12, which are together necessary and sufficient. *Rps7* and *Rps10* coincide with *Mla* barley powdery mildew and *rpg4/Rpg5* stem rust resistance loci, respectively. These discoveries establish a shared genetic architecture of multiple pathogen recognition to adapted and non-adapted pathogens and indicate a complex interplay shaped by short-term and long-term evolutionary processes.

[O57] Durable and Non-Durable Resistance: Bridging the Gulf between Academia and Agriculture

James K.M. Brown

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, U.K.

Correspondence to: james.brown@jic.ac.uk

Academic research on disease resistance in crops focusses strongly on major recognition genes although their benefit is usually ephemeral. By contrast, the performance of UK cereal varieties over the last 25 years indicates that breeders have achieved high polygenic, quantitative resistance to most diseases including rusts, mildew and Septoria. A few varieties with resistance which has 'broken down' have been released but for mildew and Septoria especially and largely for rusts, durable resistance has prevented serious crop losses. Breeders are increasingly accumulating durable, quantitative resistance to most or all significant diseases in most varieties. This was even the case during the population replacement of *Puccinia striiformis* f. sp. *tritici* in the UK between 2010 and 2014. This is in striking contrast to the preoccupation of academia with major genes which are inherently not durable. Quantitative resistance may affect other desirable plant traits, but small trade-offs can be mitigated by breeding. Two significant challenges for increasing quantitative resistance are how to exploit the wide range of genetic variation in germplasm resource collections, and how to increase the speed and efficiency of selection in field trials; technology such as machine learning may generate advances in both areas. Coevolutionary theory predicts that major genes will be less ephemeral when there is greater ecological diversity but disseminating experience of how to breed for quantitative resistance may be a more practical option for feeding a largely urban population.

[O58] Advances in wheat breeding: Meeting the challenges of rust resistance

Urmil Bansal

Plant Breeding Institute, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Cobbitty, New South Wales 2570, Australia

Correspond to: Urmil.bansal@sydney.edu.au

Rust diseases, caused by *Puccinia* species, are among the oldest and most destructive threats to wheat production. The high evolutionary potential of rust pathogens often outpaces plant breeders,

rendering resistance genes ineffective shortly after the release of new wheat cultivars. To achieve long-term control of these pathogens and ensure global food security, it is essential to strategically deploy combinations of resistance genes and continuously monitor pathotypic variation. Significant progress has been made in breeding wheat with rust resistance, with the ongoing identification and characterization of genetically diverse resistance sources being central to the fight against these pathogens. The use of advanced genomic techniques, such as next-generation sequencing (NGS), has enabled the sequencing of the whole wheat genome and the development of many single nucleotide polymorphism (SNP) arrays (e.g., 9K, 90K, 630k etc). These genomic resources have accelerated the discovery of new sources of resistance and also facilitated the cloning of several rust resistance genes over the past two decades. Techniques like genome-wide association studies (GWAS) and AgRenSeq have also helped identify new genomic regions associated with rust resistance. The progress in genomic information has paved the way for genomic selection in wheat. This talk will explore the historical developments in genomic technologies and their application in breeding rust-resistant wheat cultivars more efficiently to meet the needs of the growing global population.

[O59] Western Hemisphere Wheat Powdery Mildew Reveals Surprises

Christina Cowger^{1,2}, Rebecca Whetten¹, and Tim Kloppe²

¹Plant Science Research Unit, US Department of Agriculture-Agricultural Research Service, North Carolina State University, Gardner 3409, Raleigh, North Carolina 27695, USA; ²Department of Entomology & Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695, USA
Correspondence to: Christina.Cowger@usda.gov

As expected, *Blumeria graminis* f. sp. *tritici* (*Bgt*) populations in the U.S., Canada and Brazil are descended from ancestral Eurasian populations. However, interesting anomalies have arisen in *Bgt* populations of the Western Hemisphere. For example, a novel virulence allele was found in the *AvrPm1a* effector that interacts with the *Pm1a* resistance gene in wheat; this allele was not detected in *Bgt* populations outside North America. Moreover, a second effector that also interacts directly with *Pm1a* was found on a different *Bgt* chromosome, indicating a “genes for gene” relationship in which virulence mutations at both loci are necessary to overcome *Pm1a*. This rare situation helps explain the unusual durability of *Pm1a* resistance in commercial North American wheat production. Another interesting anomaly is the high level of virulence complexity found in Brazilian *Bgt*. Powdery mildew resistance has not historically been a prioritized trait for breeders targeting the Brazilian wheat market. Nevertheless, isolates from Brazil were virulent to more low-numbered *Pm* genes (*Pm1-Pm17*; 85%) than were isolates originating in the Fertile Crescent, Russia or the USA (50-65%). The Brazilian *Bgt* population also appears to have a small effective population size, yet wheat powdery mildew epidemics have worsened there in the past decade. Taken together, these clues suggest a genetic bottleneck followed by emergence of a broadly virulent and aggressive Brazilian *Bgt* population. The unusual interactions between powdery mildew and wheat in the Western Hemisphere illustrate the new directions pathogen populations can take in the global host-pathogen periphery.

[O60] Safeguarding wheat yields from cereal fungal invaders

Swathy Puthanvilla-Surendrababu¹, Sarah Bailey¹, Loizos Savva¹ and [Diane G. O. Saunders](#)¹

¹John Innes Centre, Norwich Research Park, Norwich, UK, NR4 7UH

Correspondence to: diane.saunders@jic.ac.uk

Wheat rusts are known as the “polio of agriculture” due to the threat they pose to wheat production worldwide. Despite long-standing efforts by a global community to wrestle the wheat rusts into submission, new strains are constantly evolving that can overcome the barriers we create to inhibit infection. To tackle these re-emergent threats, in our lab we use various genomics-based approaches to enhance the resilience of our wheat production system. For instance, developing new strategies to identify plant genes targeted by wheat rust pathogens to support successful colonisation of wheat. Disrupting the function of several of these genes has shown that their function is essential for supporting wheat rust infection. This includes the branched-chain amino acid transferase-1 (*TaBCAT1*), where disruption mutants have elevated BCAAs and salicylic acid (SA), thereby priming defence responses and significantly reducing wheat rust infection. Taking this further, we also recently deciphered the previously unknown link between BCAA accumulation and SA induction, finding that the function of the SIZ1 SUMO E3 ligase plays a critical role in this process. Another gene we uncovered encodes isocitrate lyase (*TaICL*), where disruption mutants accumulate aconitic acid that can also act exogenously to suppress wheat rust infection. Preliminary growth and development analysis of *TaBCAT1* and *TaICL* disruption mutants in glasshouse conditions, has suggested performance comparable to wild type plants. Thus, these genes present as promising new targets for manipulation in wheat rust resistance breeding that could help in our battle to safeguard wheat yields from these notorious fungal invaders.

[O61] Genomic basis of stripe rust resistance in global common wheat: an information-rich landscape

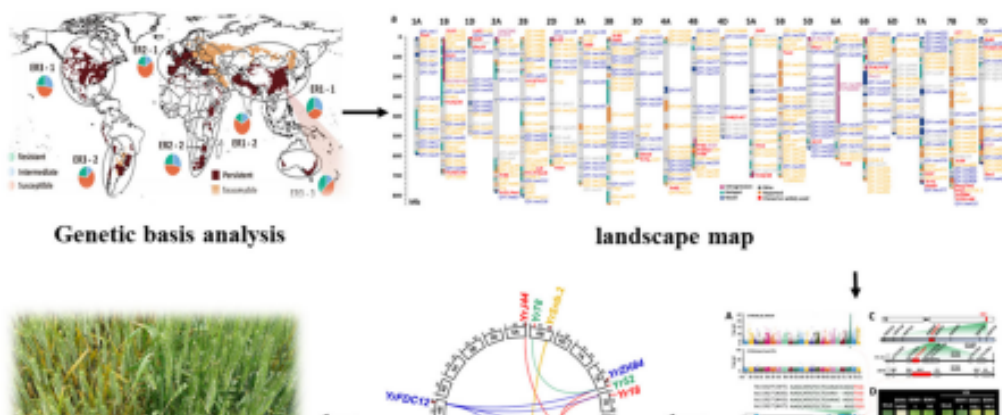
[Jianhui Wu](#)¹, Shengwei Ma², Jianqing Niu², Weihang Sun¹, Haitao Dong¹, Jiwen Zhao¹, Shengjie Liu¹, Qingdong Zeng³, Xiaojie Wang³, Dejun Han¹, Hongqing Ling², and Zhensheng Kang³

¹State Key Laboratory of Crop Stress Resistance and High-Efficiency Production, College of Agronomy, Northwest A & F University, Yangling, Shaanxi, P. R. China, 712100; ²Yazhouwan National Laboratory, Sanya, Hainan, P. R. China, 572024; ³State Key Laboratory of Crop Stress Resistance and High-Efficiency Production, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, P. R. China, 712100

Correspondence to: wujh@nwafu.edu.cn; kangzs@nwafu.edu.cn

Wheat (*Triticum aestivum* L.) suffers severe losses to yellow rust (YR) caused by the globally widespread fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). Lack of comprehensive information on genetic resistance limits knowledge-guided global deployment of resistance (R) genes in breeding programs. Here, combining 2,191 wheat genomes worldwide with over 47,000 YR responses datapoints, we revealed geographic variation in the genetic basis of YR resistance and established a reference genome-wide landscape of 559 candidate genes distributed across 431 genomic loci. Such a large-scale dataset not only allowed a comprehensive understanding

of YR resistant loci from the historical and geographical aspects, but more importantly, provided a wealth of novel genetic resources for future breeding. Notably, using fine-mapping, mutagenesis, gene silencing and genetic transformation, we cloned a series of substantial R genes with new function alleles. *Yr5x* is effective against multiple *Pst* races, *Yr6* provides resistance to YR and powdery mildew, *Yr9* shows effectiveness in some field environments, and *YrKB* (*TaEDR2-B*) confers broad-spectrum rust resistance without yield penalty. Moreover, we suggest prediction models for breeding cultivars with improved stripe rust resistance. Our findings pave new ways to support efforts for engineering favorable alleles for use in breeding for wheat durable disease resistance.



[O62] Protein-protein network hubs in host-pathogen interactions: Targets for next-generation breeding

Roger P. Wise^{1,2}, Valeria Velásquez-Zapata², Shivansh Patel³, Schuyler Smith^{3,4}, John Higgins^{3,4}, Zoi Goebel³, Gregory Fuerst¹, and J. Mitch Elmore⁵

¹USDA-Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011; ²GreenLight Biosciences, Inc. Research Triangle Park, NC 27709;

³Department of Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA 50011; ⁴Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831; ⁵USDA-

Agricultural Research Service, Cereal Disease Laboratory, 1551 Lindig Street, St. Paul, MN 55108

Correspondence to: Roger.Wise@USDA.gov

Disease phenotypes are the result of dynamic changes in gene and protein interactions at multiple levels in multiple cellular compartments. To establish a regulatory network view of protein-protein interactions (PPI) critical to pathogen infection and disease resistance in cereals, we constructed PPI networks of barley (*Hordeum vulgare* L.) in response to powdery mildew, caused by the ascomycete fungus, *Blumeria hordei* (*Bh*). The barley MLA nucleotide binding, leucine-rich repeat (NLR) receptor was used as a model regulator to interrogate host immune response, as its alleles and orthologs confer recognition specificity to diverse fungal diseases, including powdery mildew, stem rust, stripe rust, spot blotch, and rice blast. On the pathogen side, 48 representative *Bh*

effector proteins, including AVR_{A1}, AVR_{A6}, AVR_{A7}, AVR_{A9}, and AVR_{A13}, were selected from time-course RNA-sequencing on barley CI 16151 progenitor and fast-neutron immune mutant derivatives. Next, all MLA domains and *Bh* effectors were used as baits in triplicated yeast two-hybrid next-generation interaction screens, where batch matings with a 3-frame infection prey library were followed by quantitation and ranking of individual Illumina 20-million read samples via project-developed NGPINT and Y2H-SCORES software, and subsequent binary confirmation. Results were integrated with the HvInt barley interactome, enabling assembly of a high-confidence host-pathogen network of 1085 proteins and 1497 interactions to further probe cellular localization and immune activation for next-generation breeding to new and emerging pathogens.

[O63] Life, the universe, and everything for \$42: WideSeq mapping of mutants

Rajdeep S. Khangura¹, Phillip San Miguel², Amanpreet Kaur^{3,4}, Brian P. Dilkes^{3,4}

¹Department of Plant and Agroecosystem Sciences, University of Wisconsin, Madison, WI 53706, USA; ²Genomics Facility, Purdue University, West Lafayette, IN 47097, USA; ³Center for Plant Biology, Purdue University, West Lafayette, IN 47097, USA; ⁴Department of Biochemistry, Purdue University, West Lafayette, IN 47097, USA

Correspondence to: rkhangura@wisc.edu

Convenient and economical genotyping methods and simplified bioinformatic workflows are critical for genetic studies and breeding. The declining cost of short-read sequencing, innovative library preparation methods, and multiplexing have enabled low-cost, large-scale genetic and genomic studies. Here, we present an uncomplicated method for skim sequencing and amplicon sequencing with bioinformatics pipelines sufficient for various genotyping and gene expression applications. The method nicknamed WideSeq, costs \$21 (USD) and provides ~100k paired-end reads per sample when performed at scale. Input samples for WideSeq can be genomic DNA samples or double-stranded PCR products. The lower data throughput from WideSeq permits bioinformatic analysis on a regular laptop or desktop, which can aid in bioinformatic training and introduction to genomic analysis for students and first-time genomicists. Using whole-genome maize DNA samples derived from multiple pedigreed populations, including advanced backcrossed progenies, mapping of dominant and recessive mutants, bi-parental populations, near-isogenic lines, and recombinant inbred lines, we demonstrate that this genotyping method can map loci, detect donor introgressions, identify haplotypes, and perform allele-specific expression. Mutants can be mapped as single introgressions for \$21 or by comparing mutant and wild-type siblings for \$42. Interestingly, we can even perform mapping-by-sequencing to detect donor genomes derived from parents of unknown origin and map classical mutants of unknown pedigree without prior marker discovery. Location can be further refined following haplotype analysis and re-calculation of allele frequencies. This approach can be implemented using commercially available sequencing services at a moderate cost increase.

[O64] Global Genomic Landscape of Wheat-Wheat Rust Interactions

Eduard Akhunov^{1,2}, Jason Zurn^{1,2} Ural Yunusbaev^{1,2}, Evgenii Liakh^{1,2}, Zitong Yu^{1,2}, Fei He^{1,2}, Wei Wang^{1,2}, Thomas Ingram⁴, Buket Sahin^{1,2}, Alina Akhunova^{1,3}, Xianming Chen⁵, Matthew Moscou⁴, Mathew N. Rouse⁴

¹Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA; ²Department of Plant Pathology, Kansas State University, Manhattan, KS, USA; ³Integrated Genomics Facility, Kansas State University, Manhattan, KS, USA; ⁴USDA-ARS, Cereal Disease Lab, St. Paul, MN, USA; ⁵USDA-ARS, Pullam, WA, USA.

Correspondence to: eakhunov@ksu.edu

A comprehensive characterization of the resistance (R) gene network in wheat is essential for safeguarding global wheat production. To better understand the R-gene landscape, a global panel of 400 spring wheat lines was evaluated with 148 *Puccinia graminis* f. sp. *tritici* (Pgt) and 20 *P. striiformis* f. sp. *tritici* (Pst) isolates from diverse geographic regions. Association mapping using SNPs and k-mers identified 139 and 77 unique haploblocks carrying distinct *Sr* and *Yr* genes, respectively. Most genes were novel, including seven effective against the Ug99 lineage of Pgt. Long-read genome assemblies representing resistant haplotypes in the panel, integrated with RNA-seq and k-mer data, were used to identify candidate *Sr* genes. Multiple candidates were located within structural variants and ancient introgressions from wild relatives. To accelerate R gene discovery, we developed a high-throughput virus-based method for candidate gene editing. We demonstrate this approach through rapid validation of the *Sr28* allele in line GF361 using whole-genome sequencing. To edit candidate genes, 40 gRNAs were delivered via a virus vector into F1 hybrids between GF361 and a Cas9-expressing donor, targeting 13 candidates in the QTL region. Mutant screening identified a single edited NBS-LRR gene whose disruption resulted in susceptibility to Ug99. By integrating R gene maps based on screening diverse pathogen isolates with whole-genome assembly/annotation of resistant lines and high-throughput genome editing, this project aims to build a detailed catalog of wheat R genes to advance understanding of rust immunity and accelerate crop improvement through biotechnology and breeding.

[O65] Wheat breeding for durable rust resistance in the Canadian Prairies

Santosh Kumar¹, Jasdeep Kaur¹, and Brent McCallum¹

¹Brandon Research and Development Centre, Agriculture and Agri-Food Canada, 2701 Grand Valley Road, Brandon, Manitoba, R7A5Y3; ²Morden Research and Development Centre, Agriculture and Agri-Food Canada, Route 100, Unit 100-101 Morden, Manitoba, R6M1Y5

Correspondence to: Santosh.kumar@agr.gc.ca

Canadian wheat research is a multifaceted effort aimed at improving yield, quality, and disease resistance. In 2024, Canada's total wheat production rose by 6.1% year over year to 35.0 million tonnes with spring wheat production rising by of 2.2% to 26.1 million tonnes. Canada has been at the forefront of wheat rust research to combat and prevent rust epidemics. Our breeding program has stacked various combinations of rust resistant genes *Lr2a*, *Lr13*, *Lr16*, *Lr21*, *LrZH22*, *Lr27*, *Lr34*, *Lr37*, *Lr68*, *Lr74*, *Lr80*, and *Lr82*. The varietal gene combinations include *Lr34* for broad spectrum

and up to 7 additional race specific genes to provide long lasting rust resistance in uniform and stable Canada Western Red Spring germplasm. To identify and understand additional sources of rust resistance, a biparental mapping population of 252 lines were developed from a cross between AAC Wheatland/PT485. The leaf rust incidence and severity was analyzed for two years (2023-2024) in an inoculated leaf rust nursery using epidemic races prevalent in western Canada between 2022 and 2023. The mapping population was genotyped using the 25K SNP chip through SGS, North America (US). The genotypic data was curated (quality control, genotype calling, imputation) and the linkage map was constructed in MSTmap software using 4552 SNP. The QTL analysis was performed on Qgene (4.4.0) software. Three quantitative trait loci (QTL) were identified on linkage groups 5, 6 and 28 corresponding to chromosomes 1, 5 and 7 respectively. The presentation will describe the genomic loci with genes of interest.

[O66] Developing Cisgenic Resistance Gene Stacks for Improved Resistance to Wheat Stem Rust Disease

Ming Luo¹, Aihua Wang¹, Dhara Bhatt¹, Brenton Brooks¹, Brian Steffenson², Taj Arndell¹, Melania Figueroa¹, Peter Dodds¹, and Mick Ayliffe¹

¹CSIRO A&F, GPO Box 1700, Canberra, Australia; ²Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA

Correspondence to: mick.ayliffe@csiro.au; ming.luo@csiro.au

Fungal rust diseases are a major production constraint in wheat with the combined annual global cost of stem rust, stripe rust and leaf rust estimated to be more than \$3 billion. The most cost effective and environmentally sustainable approach to control rust diseases is via genetic resistance. However, these pathogens evolve rapidly and can quickly overcome resistance genes, particularly when they are deployed individually. Polygenic resistance is believed to provide more durable resistance, however selection of multiple unlinked resistance genes in breeding programs is difficult and expensive and gene combinations quickly separate in future breeding efforts. A more effective strategy for strong, longer-lasting resistance is to combine multiple genes into a single locus with multi-gene cassettes. We have developed wheat lines containing multi-gene cassettes encoding up to five different stem rust resistance genes at single loci. Furthermore, two separate resistance gene stacks, each encoding five wheat stem rust resistance transgenes, have been combined by conventional breeding to generate wheat plants containing an unprecedented level of poly-transgenic stem rust disease resistance. Next generation stem rust resistance gene stacks that also encode 5 resistance genes have now been produced in wheat using a Precision Engineering approach that incorporates only wheat DNA sequences i.e. no selectable markers or cloning scars. These entirely cisgenic plants have the potential for reduced regulatory burden given recent changes to GM legislation in some countries. This cisgenic technology is applicable to other crops for improving disease resistance and for developing other polygenic traits of agronomic significance with single gene inheritance.

[O67] Genetic interactions that suppress or unlock rust resistance in wheat

Colin W. Hiebert¹, Jyoti S. Sharma², Matthew J. Moscou³, Wolfgang Spielmeier^{4*}, Matthew N. Rouse³, Curtis J. Pozniak⁵, Krystalee Wiebe⁵, Brent D. McCallum¹, James G. Menzies^{1*}, Andriy Bilichak¹, and Tom Fetch^{1*}

¹Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB, Canada, R6M 1Y5; ²University of Minnesota, Department of Plant Pathology, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN, USA 55108; ³Cereal Disease Lab, United States Department of Agriculture, 1551 Lindig St., St Paul, MN, USA 55108; ⁴CSIRO Plant, GPO Box 1700, Canberra ACT, Australia, 2601; ⁵Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8

*retired

Correspondence to: colin.hiebert@agr.gc.ca

Genetic resistance to rust diseases is the preferred method of disease control in wheat. While the genetics of resistance can often be relatively simple, there are instances of genetic interactions that vary in their complexity. The suppressor gene, *SuSr-D1*, suppresses stem rust resistance in Thatcher derivatives, while *Lr34* acts as a nonsuppressor of stem rust resistance. While this relationship has been known for decades, it is unknown which genes interact with *SuSr-D1* and *Lr34*, and whether they interact with the same genes. Two mapping populations, one fixed for mutant alleles of *SuSr-D1* and the other fixed for *Lr34*, were used to map the chromosome regions that contributed to stem rust resistance when *SuSr-D1* function was lost or *Lr34* was added. The results showed that there are two chromosome regions that contributed to resistance in both populations. The most significant region was on chromosome 3B and corresponded with the region carrying *Sr12*. An additional population, also fixed for *Lr34*, confirmed this region's importance to the stem rust resistance that is expressed in the presence of *Lr34*. *Sr12* was chosen as a target for further investigation. Sequencing *Sr12* mutant lines, a candidate gene was identified. Gene editing and transformation experiments are ongoing. Preliminary results from the gene editing experiments suggest that *Sr12* has been identified. Identifying *Sr12* will make it possible to determine if *Sr12* is involved in these interactions or if a novel gene interacts with *SuSr-D1* and *Lr34*.

[O68] Development of Canadian barley with inbuilt resistance to stem rust

Ana Badea¹, William G. Legge^{1^}, Tom Fetch Jr^{2^}, Donald E. Harder^{2^}, James R. Tucker¹

¹Brandon Research and Development Centre, Agriculture and Agri-Food Canada, P.O. Box 1000A, R.R. 3, Brandon, MB Canada, R7A 5Y3; ²Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Rte 100 #100, Morden, MB, Canada, R6M 1Y5; ³Plant Gene Resources of Canada, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada

[^]Retired.

*Corresponding authors: Ana Badea (ana.badea@agr.gc.ca) and James Tucker (james.tucker@agr.gc.ca)

During severe epidemics, stem rust incited by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is capable of causing complete crop loss in barley (*Hordeum vulgare* L.). Moreover, the virulent stem rust race Ug99, first identified in 1998 in Uganda, Africa, has evolved into over a dozen race variants and has spread throughout several countries of Africa and into the Middle East. More recently, virulent races have also been isolated from Europe and Pacific Northwest region of North America. In Canada, barley is a significant crop grown for malting, feed and forage (general- purpose) and food. In addition to the thousands of barley growers that derive income from barley production, barley is critical to the livestock industry, grain handlers and value-added industries, such as maltsters, brewers and the food industry. While fungicides might be able to control stem rust outbreaks, genetic resistance remains the most sustainable disease management practice. Thus, it is important to develop barley cultivars with inbuilt stem rust resistance that offer crop protection. The breeding effort at Agriculture and Agri-Food Canada, Brandon Research and Development Centre encompasses screening of gene bank germplasm, double haploid production, marker-assisted selection, and field testing in disease nurseries at Brandon, MB, Canada (*Pgt* QCCJ) and Njoro, Kenya, Africa (Ug99 mix). Progress has been made and recently, a resistant two-row general purpose line, TR20273, with good agronomic package was supported for recommendation for registration in western Canada.

[O69] Effector Recognition and Activation Mechanisms of wheat NLR and Kinase-Fusion Proteins

Jian Chen¹, Oliver R. Powell², Renjie Chen², Ming Luo¹, Li Chen Cheah¹, Renee Su¹, Megan Outram¹, Camilla Langlands-Perry¹, Andrew Warden¹, Taj Arndell¹, Michael A. Ayliffe¹, Cheryl Blundell¹, Melania Figueroa¹, Jana Sperschneider¹, Thomas Vanhercke¹, Stefan T. Arold³, Brande B. H. Wulff², Peter N. Dodds¹

¹CSIRO Agriculture and Food; Canberra, Australian Capital Territory, Australia; ²Plant Science Program, Biological and Environmental Science and Engineering Division (BESE), King Abdullah University of Science and Technology (KAUST); Thuwal, Saudi Arabia; ³Bioscience Program, Smart Health Initiative, BESE, KAUST; Thuwal, Saudi Arabia.

Correspondence to: peter.dodds@csiro.au

Most resistance genes to rusts and powdery mildews encode nucleotide-binding, leucine-rich repeat immune receptors (NLRs) that recognise pathogen effectors to trigger plant immunity. However, crops are still under constant threat as these pathogens often overcome plant immunity due to rapid evolution. Understanding how these immune receptors recognise these cognate effectors and the mechanisms of immunity activation is crucial to inform the deployment of immune receptors to manage plant disease in the field. Detailed analysis of the recognition interaction between the wheat NLR receptor Sr50, Sr33 and their cognate stem rust effectors has enabled development of strategies to engineer wheat receptors to expand their recognition profiles as a next frontier in genetic control of plant diseases. Through precise engineering, we developed a modified Sr50 protein capable of recognizing both AvrSr50 and AvrSr33, while simultaneously restoring recognition of the virulent avrSr50 effector variant B6. The identification of stem rust AvrSr62 effectors has also facilitated the functional characterization of the Sr62 tandem kinase

(Sr62TK), which belongs to an emerging class of kinase-fusion type receptors in wheat and its relatives. Sr62TK requires an NLR to confer stem rust resistance in wheat and the Sr62TK/Sr62NLR pair uses a novel activation mechanism to trigger resistance. Briefly, Sr62TK self-inhibits via physical interaction between its kinase 1 and kinase 2 domains. The cognate effector, AvrSr62, competitively binds to kinase 1 to release kinase 2, which activates the Sr62NLR by physical interaction. This may represent a guard/decoy type recognition or a novel sensor-helper interaction.

[O70] Recent long-distance dispersal events have strong impact on the global landscape of wheat yellow rust

Mogens Støvring Hovmøller^{1*}, Tine Thach^{1*}, Julian Rodriguez-Algaba¹, Jens Grønbech Hansen¹, Kumarse Nazari², David Hodson³, Marcel Meyer⁴, Robert F. Park⁵, and Annemarie Fejer Justesen¹.
¹Global Rust Reference Center, University of Aarhus, Department of Agroecology, Forsøgsvej 1, DK4200 Slagelse, Denmark; ²Turkey-ICARDA Regional Cereal Rust Research Center, Izmir, Turkey; ³CIMMYT Nepal, Khumaltar, Lalipur, Nepal; ⁴Crop Science Group, INRES, University of Bonn; ⁵Plant Breeding Institute, School of Life and Environmental Science, Faculty of Science, The University of Sydney, Narellan, NSW, Australia.

*Shared 1st authorship

Correspondence to: mogens.hovmoller@agro.au.dk

The establishment of the Global Rust Reference Center (GRRC) in 2008 (www.wheatrust.org) coincided with the initiation of the most comprehensive international wheat rust surveillance efforts ever, facilitated by the Borlaug Global Rust Initiative and generous donors. Together with additional European and bilateral initiatives, this allowed us to develop a unique dataset comprising validated SSR genotyping results of more than 3200 *P. striiformis* samples collected between 2009 and 2023 from 41 countries on six continents, thereby providing a unique opportunity to study the spread of an important crop pathogen at the global scale. Our dataset demonstrates unique inter-continental dispersal events of the yellow rust pathogen in more than 10 cases, providing evidence of recent sharing of identical multi-locus genotypes (MLGs) and pathogenic race variants between continents, e.g., Europe - South America, Europe - Australia, South Asia - East Africa, and Middle East - East Africa/Northern Europe. Further, we assessed the plausibility of wind dispersal, involving a set of trajectory simulations to develop hypotheses about the causal mechanism for co-occurrence of MLGs on different continents. Our study highlights the importance of global surveillance networks for understanding connectivity of agricultural landscapes and for mitigating risks posed by novel strains. The contributions of hundreds of collaborators world-wide assisting in submission of samples of rust infected wheat and related cereals and grasses, and the contributions from multiple lab assistants and researchers, who have served GRRC and collaborating labs during this long-term study, are greatly appreciated.

[O71] Re-parameterising wheat stem rust models to explore disease dynamics in a changing climate

Césarée Morier-Gxoyiya¹, Clare M. Lewis¹, Richard J. Morris¹, Daniel P. Bebber², Diane G. O. Saunders¹

¹John Innes Centre, United Kingdom; ²University of Exeter, United Kingdom

Fluctuations in environmental conditions driven by climate change are altering the distribution and aggressiveness of plant pathogens, posing a significant threat to future agricultural productivity due to disease-related yield losses. One pressing concern is the (re-)emergence of historically devastating crop diseases [1]. In the United Kingdom (UK), reports of wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) have recently increased, signalling a potential resurgence of this historically devastating pathogen in the region [2]. Given *Pgt*'s thermal requirements, disease development is expected to be influenced by warming temperatures. Previous efforts to model wheat stem rust epidemics have largely relied on experimental data from the last century, including foundational contributions by Dr. Alan Roelfs [3]. However, as climate conditions shift and pathogen populations adapt, there is a critical need to update these models with data from contemporary *Pgt* isolates. To address this, we conducted controlled experiments using current UK *Pgt* isolates to assess the effects of temperature and leaf wetness on key pathogen life cycle stages. Specifically, we examined *in planta* growth, latent period, and sporulation dynamics across a range of environmental conditions. Our findings provide updated estimates of cardinal temperatures and minimum leaf wetness duration for *Pgt* development (Figure 1). These parameters are now being incorporated into climate-based disease models to refine predictions of future wheat stem rust risk in the UK. By building upon the pioneering work of rust pathologists, including Dr. Roelfs, this research enhances the UK's readiness for the resurgence of this wheat disease.

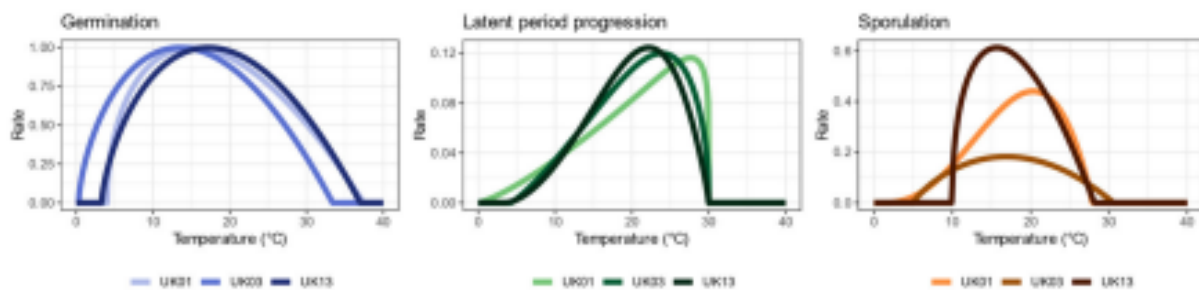


Figure 1. Modelled *Pgt* urediniospore processes vary substantially between *Pgt* isolates at different temperatures. Germination data extracted from [4].

[O72] Genomes, Germs, and Grains: an overview of 13 years of stripe rust research in 11 Canada

Gurcharn S. Brar^{1,2}

¹Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Canada

²Faculty of Land and Food Systems, The University of British Columbia, Vancouver, Canada

Correspondence to: gurcharn.brar@ualberta.ca

This talk will present an overview and lessons learned from 13 years of research (by Cereal Breeding Lab) on wheat stripe rust in Canada.

[O73] Population Genetics Structure of the South Asian *Puccinia striiformis* population

Sajid Ali^{1*}, Monsif Ur Rehman¹, Muhammad Rameez Khan¹, Aamir Iqbal¹, Muhammad Awais², Zia Ur Rehman¹, Muhammad Imtiaz³, Kerstin Flath⁴, Jerome Enjalbert⁵, Mogens Hovmøller⁶

¹Department of Agriculture, Hazara University, Mansehra, Pakistan; ²State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; ³International Centre for Agriculture Research in Dry Areas (ICARDA), Pakistan; ⁴Julius Kühn-Institut, Germany; ⁵INRAE UMR 320 Génétique Végétale, Ferme du Moulon, F-91190 Gif sur Yvette, France; ⁶Department of Agroecology, Aarhus University, Flakkebjerg, DK-4200 Slagelse, Denmark

Correspondence to: bioscientist122@yahoo.com

The Himalayan and near Himalayan region in South Asia represent the centre of diversity of *Puccinia striiformis*. We explored the spatial and temporal pathogen population structure based on a series of population studies and field experimentations, with particular emphasis on the role of host resistance. Comparison of populations from Nepal, Pakistan and Bhutan, via genotyping of 147 samples, revealed high diversity and a clear divergence across these countries. Comparison of the bordering Himalayan populations of China and Pakistan, based on 1245 samples, revealed a strong divergence over the two sides isolated by gigantic Karakoram mountains. A low pathotype diversity in genetically diverse Nepalese population was observed, where the same pathotype was represented by different genetic groups. A countrywide diversity and lack of spatial structure was observed in Pakistan, based on 1053 samples, with the detection of five distinct genetic clusters resampled across the country. Although there was no host-specific lineage(s)/structure, these diverse lineages/ MLGs were detected on a single susceptible host. The role of host was further assessed through analysing 640 samples collected on Pakistani and European wheat germplasm, which revealed the presence of host dependent pathogen divergence. We emphasize on an overall high diversity in the South Asian population with isolation across countries, explained by both distance and geographical barriers. Similar pathotype could emerge in diverse genetic lineages, while diverse lineages could infect a single susceptible variety, with impact of diverse hosts on pathogen population. The results have multiple implication for disease control though genetic resistance.

Poster Presentations

- * [P1] Assessment of Disease Screening Methods, Pathogen Strains, and Cultivar Resistance to Bacterial Blight in Highbush Blueberry
- * [P2] Isolation and Characterization of Bacteriophages for Managing Bacterial Blight (*Pseudomonas syringae* complex) of Highbush Blueberry
- * [P3] Impact of Clubroot on Microbial Communities in the Rhizosphere of Canola.
- * [P4] Characterizing the pH Sensitivity of *Verticillium longisporum*
- * [P5] Genetic Characterisation of *Puccinia striiformis* f. sp. *tritici* Isolates Suggests Southern Migration Into South Africa
- * [P6] Current situation for Swiss Needle Cast in British Columbia
- * [P7] Genetic Comparison of South African and Global *Puccinia striiformis* f. sp. *tritici* Isolates
- * [P8] Detection, Host Range, and Glyphosate Effects on *Verticillium longisporum*
- * [P9] Unraveling Resistance: Genetic Analysis of *Puccinia striiformis* f. sp. *tritici* (Stripe Rust of Wheat) Resistance
- * [P10] Enhancing Restoration Efforts: Evaluating Temperature Effects on Cr2 Resistance and Developing Field-Ready Tools for Cr2 Detection
- * [P11] Pre-plant disease risk assessment in carrot fields based on soil properties and microbial communities
- * [P12] Exploring barley's diversity to improve wheat's resistance to fungal pathogens
- * [P13] Optical density assay to determine the sensitivity of Globisporangium and Pythium isolates to mefenoxam

- *[P14] Exploring genetic diversity: Identifying novel sources of stem rust resistance for Canadian durum wheat breeding
- [P15] Preliminary proteomic analysis of oat response to *Blumeria graminis* f. sp. *avenae* infection
- *[P16] Tracking the Natural Emergence of *Verticillium longisporum* and Co-occurrence with *Leptosphaeria* spp. in Canola Fields
- *[P17] Genetic analysis of stem rust resistance in Western Canadian winter wheat
- [P18] Autoecism in the Cronartiaceae
- *[P19] *Beauveria bassiana*: A fungal endophyte suppresses clubroot on cabbage transplants, 2025
- *[P20] Development of Advanced Lentil Lines with Partial Resistance Against Race 0 of *Colletotrichum lentis* Causing Anthracnose
- *[P21] Identification of mutations associated with fungicide resistance in *Stemphylium vesicarium*
- *[P22] Assessing pathotype shifts in field populations of *Plasmodiophora brassicae*
- *[P23] A decision-support model for fungicide applications to manage *Stemphylium* leaf blight of onion
- *[P24] The largest Canadian population survey and genomic analysis of *Claviceps purpurea* to date
- *[P25] International Collaboration in Durum Wheat Breeding: Addressing Stem Rust Challenges in Ethiopia
- *[P26] Evidence of spatial divergence of *Blumeria graminis* f. sp. *tritici* pathotypes in Australia
- *[P27] Differentiating actively replicating vs. non-replicating forms of Hop Latent Viroid
- *[P28] Genomic analyses of a sexually recombining population of the wheat stem rust pathogen from Spain identifies putative avirulence loci

- *[P29] Bacterial leaf streak: Investigating the dynamics of pathogen virulence and host resistance
- *[P30] Monitoring the evolution of stripe rust in Canada over the past 40 years
- [P31] Genotypic and phenotypic changes in *Fusarium graminearum* under adaptive evolution to azole selection pressures
- *[P32] Impact of storage conditions on the viability and virulence of *Plasmodiophora brassicae* resting spores
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* [P1] **Assessment of Disease Screening Methods, Pathogen Strains, and Cultivar Resistance to Bacterial Blight in Highbush Blueberry**

Bibek Acharyaa^b, Rishi R. Burlakoti*, Someshwar R. Latchman^a, Amy Novinscak^a and Zamir Punja^b
^aScience and Technology Branch, Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, Agassiz, BC, V0M 1A0, Canada; ^bDepartment of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada

*Correspondence to: rishi.burlakoti@agr.gc.ca

Bacterial blight caused by *Pseudomonas syringae* complex (Psc) is an important disease affecting highbush blueberry in Canada. The objectives of this study were to develop disease screening methods for bacterial blight and understand the interaction of Psc strains with blueberry cultivars. These assays were conducted in controlled environment using factorial completely randomized design. Detached plant stems with flora buds were used in experiments. Two inoculation methods (bud inoculation and spray) were evaluated on four cultivars-‘Aurora’, ‘Calypso’, ‘Chandler’, and ‘Draper’. Analysis of variance (ANOVA) showed significant difference among cultivar ($P < 0.01$), but both screening assays were effective (not significant, $P > 0.05$) to induce bacterial blight. The cultivar ‘Aurora’ had the highest disease progress (relative area under disease progress curve, rAUDPC = 0.6), whereas ‘Calypso’ had the lowest (rAUDPC = 0.07). A significant positive correlation ($r = 0.97$, $P = 0.03$) was observed between two inoculation methods. In separate experiments, six bacterial isolates were inoculated in cultivars ‘Chandler’ and ‘Duke’ using the bud inoculation method. Disease progress was significantly higher in ‘Duke’ (rAUDPC = 0.48) than in ‘Chandler’ (rAUDPC = 0.35). Psc isolates also varied significantly ($P < 0.01$) in virulence with

rAUDPC values ranged from 0.37 (isolate Y.LI.1.a) to 0.51 (L.CA.1.a). Further research is ongoing to evaluate these screening assays and cultivar responses under outdoor conditions.

*** [P2] Isolation and Characterization of Bacteriophages for Managing Bacterial Blight (*Pseudomonas syringae* complex) of Highbush Blueberry**

Cassidy Ball¹, Bowornnan Chatapakul¹, Thomas Guy², Keiran Cross², Karen Fong², Ravnit Latchman³, Rishi Burlakoti³, Siyun Wang¹

¹Department of Food Nutrition and Health, University of British Columbia, Land and Food systems, 2205 East Mall, Vancouver, BC, Canada, V6T 1Z4; ²Summerland Research & Development Centre, Agriculture and Agri-Food Canada / Government of Canada, 4200 BC-97, Summerland, BC, Canada V0H 1Z0; ³Agassiz Research & Development Centre, Agriculture and Agri-Food Canada / Government of Canada, 6947 Lougheed Hwy., Agassiz, BC, Canada V0M 1A2

Correspondence to: cball01@mail.ubc.ca

Highbush blueberry production in Canada is impacted by bacterial blight caused by *Pseudomonas syringae* complex. The disease mainly cause damage to floral buds, contributing to berry yield reduction. The bacteria can survive in symptomatic and healthy plant tissues, entering the plant's apoplast causing infection. Frost damage provokes infection on plants, and cool, wet weather conditions favors disease progress. With very limited options available to manage this disease, bacteriophages (phages) are a promising option. Phages are viruses that replicate within a specific bacterial host resulting in cell lysis. This study aims to isolate lytic phages, testing their potential and destructive properties towards highly pathogenic *P. syringae*. Sixty-one phages were isolated from the environment with the vast majority isolated from sewage influents. Preliminary host range assessments against 20 pathogenic *P. syringae* strains identified 13 promising phages. Subsequent evaluation showed an average latent period of forty minutes with a burst size between eight and 341 phages per cell. Ideal storage conditions were 4°C and pH 8 with all phages showing deterioration when exposed to UV light. Cocktails composed of five phages were tested against highly prevalent *P. syringae* strains to determine effectiveness. Phage cocktails were applied to infected blueberry leaves and observed over a 15-day period at 10°C, resulting in bacterial reduction after 12 hours until 15 days. The results of this study show bacteriophages as a promising biocontrol to preventing bacterial blight. Further *in planta* assays are required for the efficacy of these bacteriophages to suppress the bacteria inside the plant host.

*** [P3] Impact of Clubroot on Microbial Communities in the Rhizosphere of Canola.**

McKenna Campbell¹, Mary Ruth McDonald¹, and Bruce D. Gossen²

¹University of Guelph, Department of Plant Agriculture, 50 Stone Rd E, Guelph, ON N1G 2W1, Canada; ²Agriculture and Agri-Food Canada, Saskatoon Research & Development Centre, 107 Science Place, Saskatoon, SK S7N 0X2, Canada.

Correspondence to: mrmcdona@uoguelph.ca

Canola (*Brassica napus* L.) is the largest-acreage oilseed crop in Canada, with nearly 9 M ha of canola seeded in 2024, generating over \$43 billion in economic activity. The canola rhizosphere hosts a diverse microbiome consisting of different groups of fungi, bacteria, and oomycetes. The rhizosphere microbiome likely plays an important role in plant health, including defense against soil-borne plant pathogens. One such pathogen, the obligate, biotrophic Chromist *Plasmodiophora brassicae* Woronin, causes clubroot in brassica crops. It infects canola roots, causing characteristic clubbing symptoms that impair nutrient and water uptake, leading to stunting, wilting, and plant death. In this study, the microbial communities in the rhizosphere of infected and non-infected canola roots were compared with bulk soil samples from the same location. Samples were collected in September 2024 from commercial canola fields in northern Ontario near New Liskeard, ON. rhizosphere soil was extracted using phosphate-buffered saline. Genomic analysis was performed using universal primers for 16S rRNA (bacteria and archaea), ITS (fungi), and 18S rRNA (oomycetes) to identify microbial sequences. Infected roots had higher proportions of several orders of bacterial phytopathogens such as Pseudomonadales and Xanthomonadales compared to non-infected and bulk soil samples. The composition of several orders of plant pathogenic fungi, including Erysiphales and Eucoccidiorida, was also higher in the infected root rhizosphere. There were no differences among oomycete orders. Metabarcoding results indicated that infected rhizosphere samples had lower species diversity in all three domain classes compared to non-infected rhizosphere or bulk soil.

[P4] Characterizing the pH Sensitivity of *Verticillium longisporum

Karla Flores Carrera, Jorge Cordero-Elvia, Stephen E. Strelkov, and Sheau-Fang Hwang
Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB T6G2P5, Canada Correspondence to: sh20@ualberta.ca

Verticillium longisporum causes Verticillium stripe, an emerging disease of canola (*Brassica napus* L.) in Canada. Environmental factors such as temperature, humidity, and soil pH can influence the growth and pathogenicity of soilborne pathogens. This study aimed to characterize the pH sensitivity of 88 *V. longisporum* isolates collected from Manitoba, Saskatchewan, and Alberta. The isolates were cultured in vitro on potato dextrose agar (PDA) adjusted to pH 4.7, 5.5, 6.5, 7.4, and 8.6. Colony diameter was measured after 14 and 21 days of growth. Analysis of variance (ANOVA) revealed significant differences among pH treatments ($p \leq 0.05$). At 14 days, the largest mean colony diameter was observed at pH 4.7 (34.7 ± 3.18 mm), and the smallest at pH 8.6 (29.9 ± 3.10 mm). A similar trend was observed at 21 days, with the greatest growth at pH 4.7 (52.1 ± 5.0 mm) and the least at pH 8.6 (44.0 ± 4.73 mm). Redundancy analysis (RDA) indicated that pH significantly influences growth patterns among isolates. Based on these findings, 12 isolates with contrasting responses to pH were selected for further evaluation. Six isolates exhibited reduced growth under alkaline conditions, while another six showed minimal variation across pH levels. These isolates are currently being evaluated further in planta under greenhouse conditions to determine the effects of pH on Verticillium stripe severity. Findings from this study will contribute to improved

understanding of pH-mediated responses in *V. longisporum* and support the development of more targeted disease management strategies.

*** [P5] Genetic Characterisation of *Puccinia striiformis* f. sp. *tritici* Isolates Suggests Southern Migration Into South Africa**

Amy Coetzer¹, Botma Visser¹, Tarekegn G. Terefe², Isabella du Toit¹, Zacharias A. Pretorius¹, and Willem H. P. Boshoff¹ ¹Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9301, South Africa; ²Agricultural Research Council - Small Grain, Bethlehem 9700, South Africa

Correspondence to: amycoetzera@gmail.com

In September 2022, localised outbreaks of *Puccinia striiformis* f. sp. *tritici* (Pst) occurred on irrigation wheat in the northern provinces of South Africa (SA). Race typing confirmed the first occurrence of Pst race 142E30A+ in the country. This race was first detected in Zimbabwe in 2018 and again in August 2022. Race 142E30A+ differs from existing SA races in virulence to Yr3a, Yr4a, Yr9, and Yr27, of which the latter two have been confirmed in local wheat germplasm. In this study, 16 microsatellite markers were used to genetically analyse 94 Pst isolates collected in SA and Zim between 1996 and 2024. The results revealed a low number of alleles, low gene diversity, and low allelic richness, with the average HO value being higher than HE, thereby confirming clonal reproduction. A clone-corrected data set (49 isolates) diverged into three subgroups using DARwin and STRUCTURE with minimal admixture between the subgroups. Isolates from Pst races 6E16A-, 7E22A-, 6E22A-, and 6E22A+ were divided into two subgroups (8 and 24 isolates, respectively), while the third subgroup consisted exclusively of race 142E30A+ isolates (12 SA and 5 Zimbabwe). This division was supported by a significant positive FST value with the highest variation observed between the three subgroups. The results confirmed the close genetic relationship between isolates of race 142E30A+ collected from SA and Zimbabwe. The timelines of the stripe rust outbreaks further suggested Zimbabwe as the likely inoculum source to SA, further emphasizing the importance of regional surveillance.

*** [P6] Current situation for Swiss Needle Cast in British Columbia**

Annie Dicaire¹, David Rusch², and Richard Hamelin¹

¹Department of Forest & Conservation Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada, V6T 1Z4; ²Ministry of Forests, Lands and Natural Resource Operations, 1520 Blanshard St, Victoria, BC, Canada, V8T 4J1

Correspondence to: anniedi@student.ubc.ca

Over the last three decades, Swiss needle cast (SNC) has led to hundreds of thousands of acres with moderate to severe needle loss and reduced Douglas-fir growth in the Pacific Northwest. SNC is caused by *Nothophaeocryptopus gaeumannii*, whose fruiting bodies block stomata, leading to premature needle cast and reduced growth. Previous research in Washington, Oregon, and California indicated the exact relationship between disease severity, needle loss, and growth loss

varies across regions and years. This project aims the study the effect of SNC within British Columbia (BC). To this aim, 43 monitoring plots were installed in BC and repeatedly measured at 5-years interval from 2017 to 2024. At each plot, the five biggest trees were measured for diameter at breast height and total height. One branch was collected from the upper, middle, and lower crown, and foliar retention was estimated for 1- to 6- year-old needles. Incidence (percentage of infected needles) and severity (percentage of infected stomata) were measured for first- and second-year needles. Results show variability in incidence, severity, and foliar retention per year. Incidence and severity increase with needle age while foliar retention decreases. The lower canopy had the highest incidence, severity, and total foliar retention, while the upper canopy had the highest mean foliar retention. Incidence, severity, and foliar retention are higher in the inner rather than the outer crown. The similar trends in incidence, severity, and foliar retention within the canopy are unexpected and raise questions about the impact of SNC on Douglas-fir in British Columbia.

* [P7] Genetic Comparison of South African and Global *Puccinia striiformis* f. sp. *tritici*

Isolates

Isabella du Toit¹, Willem H. P. Boshoff¹, Diane G. O. Saunders², Tarekegn G. Terefe³, Zacharias A. Pretorius¹ and Botma Visser¹

¹Department of Plant Sciences, University of the Free State, Bloemfontein 9301, South Africa;

²John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK; ³Agricultural Research Council – Small Grain, Bethlehem 9700, South Africa

Correspondence to: isabelladutoit98@gmail.com

The appearance of new races of *Puccinia striiformis* f. sp. *tritici* (*Pst*) with wider virulence poses a threat to wheat production in South Africa (SA). They represent either local adaptation of existing races or foreign incursions. Isolates of *Pst* collected in SA, Lesotho and Zimbabwe were genotyped to determine their genetic relationships with a panel of international isolates. Using MARPLE diagnostics (<https://marple-diagnostics.org/>), 387 highly polymorphic gene regions were sequenced with the Oxford Nanopore MinION platform to produce a maximum likelihood tree. The 96 isolates formed two distinct genetic clades separate from most of the international isolates. Isolates of *Pst* races 142E30A+, 7E22A- and 6E22A+ grouped with isolates from Kenya (*Pst*S1) and Azerbaijan (*Pst*S2), whereas isolates of *Pst* races 6E16A-, 6E22A- and 142E30A+ grouped with an isolate from Sweden (*Pst*S4) in the second clade. While the placement of the South African races was mostly consistent with previous microsatellite marker studies, eleven isolates unexpectedly appeared in both clades despite representing the same race. For example, isolates of race 142E30A+ were split between the two clades. While re-typing of these isolates is needed to confirm their race identity, the study suggests that considerable genetic diversity exists even within the same race. These findings provide valuable information for future surveillance studies by providing a baseline reference source to identify unknown field isolates.

* [P8] Detection, Host Range, and Glyphosate Effects on *Verticillium longisporum*

Lidan Gao¹, Haitian Yu¹, Longfei Wu¹, Rudolph Fredua-Agyeman¹, Kan-Fa Chang¹, Godfrey Chongo², Ahmed Abdelmagid³, Fouad Daayf⁴, Stephen E. Strelkov¹ and Sheau-Fang Hwang¹
¹Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2P5; ²BASF Canada Inc., Saskatoon, SK, Canada, S7K 3J9; ³Agriculture and Agri-Food Canada, Morden, MB, Canada, R6M 1Y5; ⁴University of Manitoba, Winnipeg, MB, Canada, R3T 2N2

Verticillium stripe, caused by the soilborne fungal pathogen *Verticillium longisporum*, is an emerging threat to canola (*Brassica napus*) production in Canada. While the disease has been primarily reported in Manitoba, field surveys in 2023 identified infected plants in commercial canola crops across the Prairies. PCR assays revealed the presence of *V. longisporum* lineage A1/D1 in infected plant samples, and bioassays with Alberta isolates confirmed their pathogenicity. To assess the host specificity of *V. longisporum*, both greenhouse and semi-hydroponic inoculation trials were conducted on different economically important crops. Soybean and lentil were the most affected, exhibiting reduced emergence and significant height reductions. In contrast, wheat, barley, pea, and faba bean showed greater tolerance, with wheat and barley displaying no visible symptoms. Further molecular analyses and pathogen re-isolation from symptomatic host plants are underway to confirm colonization. Additionally, the potential impact of glyphosate on Verticillium stripe development was investigated under field and greenhouse conditions. While glyphosate application had minimal effects on seedling mortality, inoculated plants treated with this compound exhibited increased disease severity and reduced plant height, regardless of application frequency. These findings highlight the increasing prevalence of *V. longisporum* in western Canada, its ability to infect crops beyond canola, and the possible influence of glyphosate on disease progression. Understanding these factors is critical for developing effective disease management strategies in canola.

*** [P9] Unraveling Resistance: Genetic Analysis of *Puccinia striiformis* f. sp. *tritici* (Stripe Rust of Wheat) Resistance**

Kara Glor¹, Gurcharn S. Brar², Samuel Holden², Meng Li², and Hadley R. Kutcher¹

¹Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK; ²Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB

Correspondence to: kyq341@usask.ca

Stripe rust of wheat (*Triticum* spp.) is caused by the pathogen *Puccinia striiformis* Westend f. sp. *tritici* Erikks (*Pst*); infection by *Pst* can result in reduced kernel number per spike and grain quality. Due to the biology of the pathogen producers are limited to fungicide applications and selection of resistant varieties for stripe rust control; selection of resistant varieties is preferred. Two general types of resistance genes are available, all stage resistance (ASR) or adult plant resistance (APR) genes. APR genes are preferred as they are durable compared to ASR genes which may be broken down quickly after variety release. The objective of this study was to identify and map novel APR genes in two bi-parental crosses ('Avocet'/'CN116322' and 'Avocet'/'CN52866'). Previous work identified APR in the resistant parents 'CN116322' and 'CN52866', molecular analysis did not

identify the presence of known APR genes. Crosses were made to the susceptible parent 'Avocet' and the F₁s were selfed to generate the F₂ mapping population. The F₂ populations were inoculated with a *Pst* isolate (*W053*) under controlled environment conditions to determine resistance phenotypes. F_{2:3} families will be phenotyped under field conditions in 2025 to confirm phenotypes and determine zygosity. Preliminary analysis suggests that resistance is conferred by one dominant gene in the 'Avocet'/'CN116322' cross, and two dominant genes in the 'Avocet'/'CN52866' cross. Susceptible and resistant candidates from both populations were selected for bulk segregant analysis sequencing (BSA-seq), and the QTL-seq pipeline is being utilized to identify regions where the resistance gene(s) may be located.

*** [P10] Enhancing Restoration Efforts: Evaluating Temperature Effects on Cr2 Resistance and Developing Field-Ready Tools for Cr2 Detection**

Macy Gosselaar¹, Richard Hamelin¹, Tom Booker¹, and Monika Fischer²

¹Forest Sciences Centre, University of British Columbia, Department of Forest and Conservation Sciences, 2424 Main Mall, Vancouver, BC, Canada, V6T 1Z4; ²Biological Sciences Building, University of British Columbia, Department of Forest and Conservation Sciences & Department of Botany, 6270 University Blvd, Vancouver, BC, Canada, V6T 1Z4

White Pine Blister Rust (WPBR), caused by the invasive fungus *Cronartium ribicola*, has devastated Western White Pine (WWP; *Pinus monticola*) populations, leading to sharp declines and local extinctions. Restoration efforts depend on deploying rust-resistant stock, primarily conferred by the *Cr2* gene, which enables pathogen recognition and defense activation. However, two major challenges threaten this strategy: the emergence of a virulent *C. ribicola* pathotype (*vcr2*) that overcomes *Cr2*-mediated resistance, and potential thermolability of *Cr2* stability. While molecular assays exist to detect *Cr2*, no field-deployable tools currently allow for rapid, on-site gene validation. As a result, it remains unclear whether resistant stock is effectively deployed, resistance is stable, or if *vcr2* has spread. To address this, we will develop a multiplex qPCR assay targeting *Cr2* and *LFY*, with *LFY* serving as an internal control to estimate *Cr2* heterozygosity through relative Ct values. Primer-probe compatibility will be assessed using ΔG calculations, and PCR efficiency will be evaluated via standard curves. We will validate the assay using 150 known *Cr2*-positive needle samples with the portable Biomeme Franklin® qPCR thermocycler. To assess resistance stability and track pathogen development, we will inoculate 80 seedlings (~50/50 MGR across four families) at 15°C, 20°C, and 25°C, sampling at 1, 4, and 7 days post-inoculation for RNA-Seq and microscopy. We expect *Cr2* expression is not exclusively temperature-sensitive, though temperature may indirectly affect its function. Our assay will enable accurate tracking of *Cr2*-resistant trees, support seedling screening, monitor deployment success, and help infer *vcr2* presence when infection occurs.

*** [P11] Pre-plant disease risk assessment in carrot fields based on soil properties and microbial communities**

Umbrin Ilyas, and Mary Ruth McDonald

Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada

Correspondence to uilyas@uoguelph.ca

Cavity spot is an economically important soil-borne disease of carrots caused by several species of *Globisporangium* and *Pythium*. The disease appears as superficial dark lesions on carrot roots, reducing the marketable yield. Diagnostic tools are not available to estimate disease risk and previous research could not predict risk based on initial inoculum in the soil. It is hypothesized that specific soil properties and microbial communities in field soils are associated with lower disease development and disease risk. Bulk muck soil (organic matter 40–80%) was collected at seeding from 22 fields in the Holland Marsh, Ontario, for soil nutrient and microbial analysis from 2021 to 23. Statistical analyses were conducted in R using DESeq2, phyloseq, and Vegan. Soil collected from fields with significantly greater cavity spot severity was classified as high-risk soil, while with lower severity was low-risk. High-risk soil had higher copper (~12 ppm) and iron (~210 ppm) while low-risk soil had higher pH (~7) and calcium content (~83%). The abundance of *Flavobacterium*, *Mesorhizobium*, *Mortierella*, *Penicillium*, *Talaromyces*, *Myzocytiopsis* was significantly greater in low-risk compared to high-risk soils. *Globisporangium* and *Pythium* were more abundant in high-risk soils. Microbial community composition in low- and high-risk soils was associated with soil properties. High-risk soils had strong correlation with copper and iron. This information will assist growers make informed decisions on cultivar selection, chemical control, and avoid fields based on disease risk.

*[P12] Exploring barley's diversity to improve wheat's resistance to fungal pathogens

Bulat Islamov^{1*}, Stefanie Lück¹, István Molnár², Simon Krattinger³, Jochen Kumlehn¹, Philipp Schulz⁴, Dimitar Douchkov^{1*}

¹Leibniz Institute of Plant Genetics and Crop Research, Corrensstraße 3, Seeland OT Gatersleben 06466, Germany; ²Agricultural Institute, HUN-REN Centre for Agricultural Research, Martonvásár, Brunszvik u. 2, 2462 Hungary; ³King Abdullah University of Science and Technology, Cereal Genetics and Genomics Lab, Thuwai, Jeddah 23955- 6900, Kingdom of Saudi Arabia; ⁴Julius Kühn-Institute (JKI) - Institute for Plant Protection in Field Crops and Grassland, Stahnsdorfer Damm 81 14532 Kleinmachnow

Correspondence to: douchkov@ipk-gatersleben.de

Wheat production is significantly impacted by fungal diseases such as powdery mildew and yellow rust, both caused by highly host-adapted biotrophic pathogens. These pathogens have co-evolved with their host for millennia, developing mechanisms to evade and suppress the plant's immune response. However, their adaptations are highly specific to the host's immune system and are ineffective against the defense mechanisms of even closely related species. This creates an opportunity to leverage the intact resistance elements of related nonhost species to enhance disease resistance in wheat. As a nonhost species for wheat fungal pathogens, barley represents a vast, untapped resource for wheat improvement. In non-adapted plant-pathogen interactions,

infection outcomes are determined at the earliest stages, often without visible symptoms, making it challenging to pinpoint resistance mechanisms using traditional phenotyping methods that rely on visual assessment. To address this, we employ an innovative high-throughput micro- and macro-phenotyping platform to precisely quantify resistance-related traits and map associated genomic regions in 1,000 barley genotypes from the diversity core collection of IPK Gatersleben's Genebank and elite barley lines. This approach has allowed us to identify barley accessions with increased susceptibility to wheat yellow rust and powdery mildew, as well as genomic regions linked to resistance.

*** [P13] Optical density assay to determine the sensitivity of *Globisporangium* and *Pythium* isolates to mefenoxam**

Umbrin Ilyas, and Mary Ruth McDonald

Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada

Correspondence to uilyas@uoguelph.ca

Several species of *Globisporangium* and *Pythium* cause cavity spot on carrot. Disease management primarily relies on applications of the fungicide mefenoxam. Growers' reports in Ontario, Canada of severe cavity spot, even when using mefenoxam, suggests mefenoxam resistance may be prevalent in pathogen populations in this region. The objectives were to develop a rapid and accurate optical density (OD) assay to assess mefenoxam sensitivity of 188 isolates of *Globisporangium* and *Pythium* species collected from 13 carrot fields in Ontario from 2020 to 2023. The isolates belonged to seven species: *G. intermedium* de Bary, *G. irregulare* Buisman., *G. rostratifingens* Uzuhashi, Tojo & Kakishima., *G. sylvaticum* W.A. Campb. and F.F. Hendrix., *G. ultimum* Trow., *G. violae* Chesters and Hickman., and *P. sulcatum* R.G. Pratt and J.E. Mitch. The OD was measured at 600 nm in broth amended at different concentrations of mefenoxam, with OD readings at nine positions per well in a 96-well plate. The OD assay was validated by comparing EC50 values with a standard fungicide-amended agar medium assay using 20 representative isolates. There was a significant linear relationship ($R^2 = 0.97$, $P = <0.0001$) between the two assays. Of the 188 isolates tested, 24% exhibited <60% of mycelial growth at 5 $\mu\text{g mL}^{-1}$, 50% were inhibited by >60% at 5 $\mu\text{g mL}^{-1}$, 19% were inhibited by >60% at 1 $\mu\text{g mL}^{-1}$, and 7% were inhibited >60% even at 0.1 $\mu\text{g mL}^{-1}$. The OD assay proved to be efficient, rapid, and accurate for monitoring mefenoxam sensitivity of these oomycetes. Optical density assay to determine the sensitivity of *Globisporangium* and *Pythium* isolates to mefenoxam.

*** [P14] Exploring genetic diversity: Identifying novel sources of stem rust resistance for Canadian durum wheat breeding**

Madison Kist¹, Valentyna Klymiuk¹ and Curtis J. Pozniak¹

¹Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8

Correspondence to: madison.kist@usask.ca

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, remains a significant threat to global wheat production. The emergence of new races, such as the Ug99 lineage, highlights the need to identify and incorporate novel resistance into wheat breeding programs. This is particularly critical for Canadian durum wheat, where resistance is predominantly conferred by *Sr13*, to which virulence has already been detected. Wild wheat relatives harbour novel genes for disease resistance that can be utilized for wheat improvement. In this study, a tetraploid wheat diversity panel was screened against stem rust race DCB (pathotype TRRTF) to identify potentially novel sources of resistance. One promising accession, domesticated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) PI 94635, demonstrated strong resistance. Molecular marker analysis revealed that it does not carry any known *Sr* genes, including *Sr13*, suggesting that its resistance is novel. We crossed PI 94635 with the universally susceptible durum wheat (*T. turgidum* ssp. *durum*), cv. Rusty and phenotypic evaluation of F₂ seedlings with DCB indicated that resistance is controlled by a major gene. To further characterize this resistance, we will perform bulked segregant analysis sequencing (BSA-seq) on DNA bulks derived from F₂ plants expressing extreme resistant and susceptible phenotypes. This approach will enable the rapid identification of genomic region(s) associated with the novel resistance and ultimately, development of molecular markers for high resolution mapping. These markers will also serve as valuable tools for marker-assisted selection, facilitating the introgression of novel stem rust resistance into Canadian breeding programs.

[P15] Preliminary proteomic analysis of oat response to *Blumeria graminis* f. sp. *avenae* infection

Aleksandra Nucia¹, Katarzyna Michalak², Weronika Grzelak¹, and Sylwia Okoń¹

¹University of Life Sciences, Institute of Plant Genetics, Breeding and Biotechnology, Lublin, Poland; ²University of Life Sciences, Department of Epizootiology and Clinic of Infectious Diseases, Lublin Poland

Correspondence to: aleksandra.nucia@up.lublin.pl

Powdery mildew, caused by *Blumeria graminis* f. sp. *avenae* (*Bga*), is an important disease that significantly impacts oat yields worldwide. The mechanism of resistance to this disease has not been well identified. A proteomics approach is one way to identify proteins that involved in plant disease response. However, the proteomic response of oat to *Bga* infection remains unclear. To analyse the leaf proteome changes of oat in response to *Bga*, we compared the leaf proteins of the susceptible cultivar Fuchs and the powdery mildew resistant line Av1860 with a single resistance gene *Pm4*, at 48 hours post-inoculation. The leaf proteins were extracted using the TCA/acetone method, and the protein pattern was determined by two-dimensional electrophoresis (2-DE). Protein characterisation was performed using MALDI-TOF mass spectrometry (MALDI-TOF MS). Analyses were performed in three biological replicates. Proteins involved in the response to powdery mildew infection were identified by comparing the protein profile between resistant and susceptible samples before and after *Bga* infection. Protein spots with significant changes were selected and attributed to corresponding proteins by searching within bioinformatics databases. The results showed that there are significant differences between the analyzed samples. Results

were analyzed regarding the functional implications of the proteins identified, emphasising their putative roles in plant defence in response to oat powdery mildew infection.

***[P16] Tracking the Natural Emergence of *Verticillium longisporum* and Co-occurrence with *Leptosphaeria* spp. in Canola Fields**

N.Y. Pathirana¹, C. Bvindi¹, N.M.R. Vandendriessche¹, J. Feng² and W.G.D. Fernando¹

¹Department of Plant Science, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada; ²Alberta Plant Health Lab, Alberta Agriculture and Irrigation (AGI), Edmonton, AB, T5Y 6H3, Canada

Correspondence to: dilantha.fernando@umanitoba.ca

Verticillium stripe disease is spreading rapidly across Canada. One notable case occurred at the University of Manitoba's Ian Morrison Research Farm in Carman, in 2024 where *Verticillium longisporum* (VL) emerged and spread rapidly without any prior inoculation. VL detection across multiple sites suggests natural establishment and dispersal. This study investigated VL distribution, lineage composition and its co-occurrence with blackleg pathogens. Stubble samples were collected from four separate sites and subjected to lineage-specific polymerase chain reaction (PCR) analysis, revealing that nearly all VL isolates belong to the highly virulent A1/D1 lineage. DNA extracted from 20 representative stubble samples was analyzed using a triplex probe-based quantitative PCR assay targeting *V. longisporum* (P-VL), *Leptosphaeria maculans* (P-Lm), and *L. biglobosa* (P-Lb). The results confirmed frequent co-infection of VL and LM in most samples. In contrast, LB was detected at a lower frequency. To further explore pathogen interaction dynamics greenhouse trials are being conducted using canola genotypes *Rlm1* and *Rlm3* and *Rlm7*. Plants were inoculated with multiple VL lineages (A1D1, A1D2, A1D3, *V. dahliae*) and LM isolates carrying different Avr/avr profiles. The trials assess how resistance gene backgrounds influence disease development when both pathogens co-occur. Co-inoculation of VL (A1D1) and LM in canola (*Rlm2*, *Rlm3*, *Rlm4*, *Rlm7* background) showed a significant interaction in breaking down of the avirulence-R gene hypersensitive interaction to cause more disease. This research provides insight into pathogen co-occurrence in naturally infected canola fields and the devastation it can cause to canola production and supports the need for the development of disease management strategies.

***[P17] Genetic analysis of stem rust resistance in Western Canadian winter wheat**

Kaitlyn A. Pidherny¹, Jim G. Menzies², Colin W. Hiebert², Harwinder S. Sidhu³, Curt A. McCartney¹

¹Department of Plant Science, University of Manitoba, 222 Agriculture Building, Winnipeg, MB, Canada R3T 2N2; ²Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB, Canada, R6M 1Y5; ³Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403 1st Ave S., Lethbridge, AB, Canada, T1J 4B1

The fungal pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) is the causative agent of stem rust on wheat (*Triticum aestivum*). *Pgt* has the potential to cause significant losses to wheat production in Canada, making it an economically important disease. No stem rust epidemics have occurred

since the 1950s in western Canada due to the cultivation of resistant varieties and the eradication of *Berberis vulgaris*. Due to its destructive potential, stem rust is a Priority 1 disease for the Prairie Recommending Committee for Wheat, Rye, and Triticale, and breeding lines without stem rust resistance are unlikely to be registered for production in western Canada. The genetic basis for stem rust resistance in most Canadian wheat cultivars is not fully understood. The objective of this study is to identify the stem rust resistance genes (*Sr*) currently deployed in the winter wheat germplasm present in western Canada. The population utilized in the study consists of approximately 300 hard red winter wheats from western Canada, and 100 hard red winter wheats from the United States, eastern Canada, and Europe. The population was phenotyped for stem rust resistance in inoculated field trials and seedling assays. Field stem rust nurseries were grown at two locations, Winnipeg and Carman, Manitoba in 2022-2023 and 2023-2024. Preliminary genome-wide association study (GWAS) analyses identified quantitative trait loci (QTL) for stem rust resistance on chromosomes 1B, 2A, 2D, and 3D. The position of these QTL suggest the presence of *Sr6*, *Sr24*, *Sr31*, and *Sr38* in the population.

[P18] Autoecism in the Cronartiaceae

Leah Rettenbacher¹

¹University of British Columbia

The evolution of heteroecism in Pucciniales is a key factor in the success and diversification of the group, however many contemporary rusts have reverted to simpler autoecious life cycles. In the family Cronartiaceae, autoecious short-cycled rusts have developed as “correlated pairs” that exist alongside their heteroecious counterparts. One of these pairs is the autoecious *Cronartium harknessii* and the heteroecious *Cronartium quercuum* f. sp. *banksianae*. Most populations of *C. harknessii* exist outside the natural range of *Quercus rubra*, the telial host of *C. quercuum* f. sp. *banksianae*. It is unclear how *C. harknessii* spores would interact with telial host of their closest relative, and whether facultative heteroecism could occur. An inoculation experiment was conducted to monitor differences in the behaviour of both rusts upon aeciospore germination and host penetration on *Pinus contorta* and *Q. rubra*. Qualitative observations were made with SEM and confocal microscopy. Results showed that *C. harknessii* behaves similarly to *C. quercuum* f. sp. *banksianae* on *Q. rubra* where infection begins stomatal penetration, however infection does not progress further into the host. This behaviour is different than that of *C. harknessii* on its aecial pine host, where host penetration occurs directly through the host cuticle. These results add to the evidence of the lack of facultative heteroecism within *C. harknessii*, now thought to be fully clonal species. Further research into the source of incompatibility between *C. harknessii* and *Q. rubra* is needed to determine how the shift from heteroecism to autoecism in these species occurs.

***[P19] *Beauveria bassiana*: A fungal endophyte suppresses clubroot on cabbage transplants, 2025**

Kelly Ruigrok¹, Bruce Gossen², and Mary Ruth McDonald¹

¹Department of Plant Agriculture, University of Guelph, 50 Stone Road E, Guelph, ON, Canada N1G 2W1; ²Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada S7N 5E2
Correspondence to: mrmcdona@uoguelph.ca

Clubroot (*Plasmodiophora brassicae* Woronin) is a soil-borne disease of Family Brassicaceae with limited management strategies. *Beauveria bassiana* (Balsamo) Vuillemin is a biocontrol of insect pests that also colonizes plants as an endophyte to suppress disease and enhance growth. A growth room study was conducted to determine if *B. bassiana* could reduce clubroot on cabbage. Seedlings in plug trays were drenched with a commercial *B. bassiana* product: BioCeres or Botanigard. This was to allow time for colonization before inoculation with *P. brassicae*. Plants were transplanted into pots 6 weeks after seeding and inoculated with *P. brassicae* resting spores at 0, 1×10^5 , 10^6 or 10^7 spores mL⁻¹. Clubroot severity was assessed 6 weeks later. The disease severity index (DSI) in the absence of *B. bassiana* was 23, 58 and 87% for plants inoculated with 10^5 , 10^6 and 10^7 spores of *P. brassicae*; application of Botanigard reduced DSI to 7, 14 and 48% DSI. Colonization by *B. bassiana*, based on 1- cm² surface-sterilized leaf pieces on PDA, was 77% for Botanigard and 40% for BioCeres. The study was repeated. *Beauveria bassiana* treatments were also examined in the field on soil naturally infested with *P. brassicae*. There was low disease in all treatments and no differences in DSI. A substantial increase in plant fresh weight for Botanigard (+66%) and BioCeres (+75%) compared to no endophyte was found in cabbage in disease-free soil. Additional field trials are planned to confirm growth promotion and assess clubroot suppression.

*[P20] Development of Advanced Lentil Lines with Partial Resistance Against Race 0 of

***Colletotrichum lentis* Causing Anthracnose**

Viscarra Arellano Sarahi¹, Banniza Sabine¹, Vargas Ana¹

¹Plant Science, University of Saskatchewan, College of Agriculture and Bioresources, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8

Correspondence to: sarahi.viscarra@usask.ca

Colletotrichum lentis is the most prevalent and damaging foliar pathogen affecting lentil (*Lens culinaris*) production in Saskatchewan, reported in 92% of surveyed fields in 2019. The virulent race 0 dominates the pathogen population, for which partial resistance was identified in the wild relative *L. ervoides*; however, interspecific crosses between *L. culinaris* and *L. ervoides* are often unsuccessful due to post-zygotic incompatibility, embryo abortion, and hybrid sterility. To overcome these barriers, embryo rescue is critical to recover viable interspecific hybrids and enable the transfer of resistance alleles into cultivated backgrounds. In this experiment we adapted and optimized embryo rescue protocols for *L. culinaris* × *L. ervoides* crosses to support lentil breeding efforts targeting anthracnose resistance. Immature pods at similar stages from the resistance donor *L. ervoides* LR-66-590 and the *L. culinaris* parents CDC Grimm, CDC Nimble, IBC 194, 9372-H2-1 and 12353T-1-H14/H2-51 were harvested and cultured on two modified media following embryo rescue protocols based on Polowick & Yan (2023) and Saha et al. (2015). Embryo

age at excision, culture medium composition, and incubation conditions were evaluated and adjusted, maximizing recovery and regeneration rates. Recovered embryos are being evaluated for shoot promotion and rooting. The success of embryo rescue will determine the feasibility of transferring resistance from LR-66-590 into *L. culinaris* as the first step toward developing anthracnose race 0 -resistant lines. Ultimately, optimizing embryo rescue protocols will support the broader goal of enhancing genetic resistance in lentil and reducing reliance on chemical disease control.

[P21] Identification of mutations associated with fungicide resistance in *Stemphylium vesicarium

Julia Scicluna¹, Afsaneh Sedaghatkish¹, Emily Mcfaul¹, Bruce D. Gossen², and Mary Ruth McDonald²

¹Department of Plant Agriculture, University of Guelph, 50 Stone Rd East, Guelph, ON, Canada, N1G 2W1; ²Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada, S7N 0X2

Correspondence to: mrmcdona@uoguelph.ca

Stemphylium leaf blight (SLB) caused by *Stemphylium vesicarium* is the most common foliar disease of onion in Ontario. Management of SLB relies on repeated fungicide applications; most registered fungicides contain at least one active ingredient in FRAC groups 7 or 11. Resistance to FRAC 7 and 11 fungicides has been common in Ontario since 2018. Isolates resistant to FRAC 7 fungicides in New York frequently carried one of three mutations (C-G79R, C-H134R or C-C135R) in the gene encoding succinate dehydrogenase (*Sdh*). Resistance to FRAC 11 was associated with the G143A mutation in cytochrome *b*. In the current study, Kompetitive Allele Specific PCR (KASP) assays were designed to amplify C-G79R, C-H134R, C-C135R and G143A. The assays were tested on 70 isolates of *S. vesicarium* collected in Ontario from 2012–2023 that were previously classified as either sensitive or resistant to FRAC 7 fungicides. Resistant isolates were sequenced to detect additional mutations. C-H134R was identified in 29% of isolates, C-G79R in 7% and C-C135R in 3%. An additional mutation, C-H134N, was detected by sequencing in 23% of isolates. G143A was found in all isolates collected from 2021–2023. Onion plants were treated with fluxapyroxad and then inoculated with isolates carrying *Sdh* mutations under controlled conditions. Isolates carrying C-H134R reduced fungicide efficacy by 59% compared to wildtype isolates. C-H134R appears to be a major contributor to SDHI resistance in Ontario. KASP assays can be used to evaluate *S. vesicarium* populations for mutations early in the growing season to warn growers about fungicide resistance.

[P22] Assessing pathotype shifts in field populations of *Plasmodiophora brassicae

Juan Serna¹, Andrea Botero², Sheau-Fang Hwang¹ and Stephen E. Strelkov¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G2P5; ²Department of Biological Science, MacEwan University, Edmonton, AB, T5H 0K9

Correspondence to: strelkov@ualberta.ca

Clubroot, caused by *Plasmodiophora brassicae*, is a major threat to canola (*Brassica napus*) production in Canada. The virulence of pathogen populations can be influenced by repeated infection cycles on both clubroot-susceptible and clubroot-resistant host genotypes. These cycles can lead to changes in virulence on differential hosts and result in pathotype designations that differ from the original pathogen collections. This study aims to evaluate changes in the virulence of resistance-breaking and non-resistance-breaking field isolates, classified as pathotypes 3H and 5X, respectively, on the Canadian Clubroot Differential (CCD) set following repeated cycling on the susceptible canola cultivar 'Westar' and the resistant canola line LCF1. Inoculations are being conducted at different inoculum concentrations with varying ratios of each pathotype over four cycles. In addition to evaluating virulence shifts, SNaPshot assays will be used to confirm the presence and relative abundance of each pathotype *in planta*. In a preliminary greenhouse trial, seven-week-old seedlings of LCF1 and another clubroot-resistant canola genotype, '45H29', were inoculated with 1×10^7 spores per plant of an isolate pathotyped as 3H. No significant differences in disease severity index (DSI) were observed between the two resistant genotypes. Concurrently, three field isolates identified as potential resistance-breaking pathotype 5X were characterized using a subset of the CCD, with each isolate exhibiting distinct virulence profiles. This research will enhance the understanding of *P. brassicae* population dynamics and support more accurate pathotype screening for clubroot-resistance breeding in canola.

*[P23] A decision-support model for fungicide applications to manage

Stemphylium leaf blight of onion

Julia Scicluna¹, Michael Kooy¹, Bruce D. Gossen¹, and Mary Ruth McDonald¹

¹Department of Plant Agriculture, University of Guelph, 50 Stone Rd East, Guelph, ON, Canada, N1G 2W1; ²Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada, S7N 0X2

Correspondence to: mrmcdona@uoguelph.ca

Stemphylium leaf blight (SLB) caused by *Stemphylium vesicarium* is a major foliar disease of onion in the Holland Marsh, Ontario. Management of SLB relies on repeated fungicide application each season, which has contributed to high levels of fungicide resistance in the pathogen population. Decision-support models may reduce fungicide applications and resistance risk, but existing models rarely reduce fungicide applications or SLB severity in Ontario. Field trials were conducted in the Holland Marsh from 2021–2024 to assess decision-support models. A new model, STEMcast, was developed from the TOMcast model to be more specific for SLB. Triggering fungicide applications using thresholds of conidia captured by Rotorod spore traps was also assessed. There were no differences between the nontreated control and any of the treatments from 2021–2023, which was likely due to poor fungicide efficacy as a result of fungicide resistance in the pathogen. In 2024, a different fungicide combination was used and the TOMcast model reduced SLB severity compared to the nontreated control, but triggered more fungicide applications than the calendar spray program. The STEMcast model and conidia thresholds

reduced fungicide applications, but did not affect SLB severity. A controlled environment study was conducted to assess temperature and leaf wetness combinations conducive for infection by *S. vesicarium*. Temperatures of 23°C with long periods of leaf wetness were more favourable for infection than 13°C or 18°C. These results are being incorporated into the STEMcast model to further improve efficacy.

***[P24] The largest Canadian population survey and genomic analysis of *Claviceps purpurea* to date**

Skylar Shaw^{1,2}, Jim Menzies³, Miao Liu⁴, David Overy⁴, Samia Berraies⁵, Curtis Ponziak⁶, Harmeet Chawla², Sheryl Tittlemier¹, and Sean Walkowiak^{1,2}

¹Canadian Grain Commission, Grain Research Laboratory, Richardson Center for Food Technology Research, 200 Innovation Dr, Winnipeg, MB, Canada, R3T 6B6; ²University of Manitoba, Department of Plant Science, 66 Dafoe Road, Winnipeg, MB, Canada, R3T 2N2; ³Agriculture and Agri-Food Canada, Morden Research and Development Centre, Route 100, Unit 100-101 Morden, Canada, R6M 1Y5; ⁴Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 3701 Carling Ave, Ottawa, ON, Canada, K1A 0C6; ⁵Agriculture and Agri-Food Canada, Swift Current Research and Development Centre, 1 Airport Rd, Swift Current, SK, Canada, S9H 3X2; ⁶University of Saskatchewan, Crop Development Centre, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8
Correspondence to: shaws347@myumanitoba.ca

Claviceps purpurea (Fr.) Tul. is a fungal pathogen that infects cereal grains and grasses, forming growth of dark- coloured ergot sclerotia in place of normal seed or grain. These sclerotia contain alkaloid mycotoxins that are produced due to expressions of the ergot alkaloid synthesis (EAS) genes. These metabolites have been linked to both serious health detriments and potential medicinal remedies. In this study, we explore thousands of ergot sclerotia from infected cereal grains obtained from the Canadian Grain Commission’s Harvest Sample Program, as well as historical reference samples, to examine the core and dispensable genome of *C. purpurea* on a larger scale than previously performed and construct a pan-genome. Ergot sclerotia were catalogued and selected for culturing based on the province and year of origin. As *Claviceps* are obligate biotrophs that grow slowly in culture, methods were developed and optimized to harvest mycelia for DNA extraction and testing. The generation of long-read genome assemblies using PacBio Hi-Fi whole genome sequencing, and comparative analysis is performed using 70 representative gDNA samples. TaKaRa Next-Gen genotyping will be performed on thousands of sclerotia samples from the 2014-2024 harvest seasons across Canada to explore variation in the EAS gene cluster and additional gene targets potentially associated with disease and population structure. The findings of this project provide further insight into geographic, environmental, and genotypic variance of *C. purpurea*, leading to improved developments in agricultural management of disease and potential health risks.

*[P25] International Collaboration in Durum Wheat Breeding: Addressing Stem Rust

Challenges in Ethiopia

Yewubdar Shewaye¹, Ashenafi Gemechu¹, Bekele Geleta², Ayele Badebo², Zerihun Tadesse³, and Karim Ammar³

¹Ethiopian Institute of Agricultural Research (EIAR) at DZARC, Addis Ababa, Ethiopia; ²International Maize and Wheat Improvement Center (CIMMYT), Addis Ababa, Ethiopia; ³International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico

Correspondence to: yewb21@gmail.com

Durum wheat is a staple crop in Ethiopia, cultivated for centuries across diverse agro-ecological zones. Its production is vital for food security and economic development. In recent years, collaboration between the Ethiopian Institute of Agricultural Research (EIAR) and the International Maize and Wheat Improvement Center (CIMMYT) has strengthened wheat breeding, resulting in high-yielding, disease-resistant varieties. In 2024, a total of 2,466 durum wheat genotypes from CIMMYT were evaluated at the Debre Zeit Agricultural Research Center (DZARC) for resistance to stem rust, a major threat to wheat production. The study aimed to identify genotypes with durable resistance. Disease severity frequencies revealed that 49% of the lines exhibited a resistance response (severity <20%) to the local races, while 26.4% (severity range 20–35%) and 23.6% (severity range 40–60%) of the genotypes demonstrated moderate resistance to moderate susceptible responses, respectively. The remaining 1.2% of the genotypes were susceptible (severity >60%). In comparison with historical data, the resistance frequency of the CIMMYT durum wheat lines is increasing over time. The Area under Disease Progress Curve (AUDPC) analysis further confirmed that resistant genotypes had lower AUDPC values, indicating slower disease progression. These findings highlight the potential of CIMMYT's germplasm in providing durable resistance to stem rust, contributing to enhanced wheat productivity in Ethiopia. This research underscores the significance of international partnerships, such as those between CIMMYT and EIAR (DZARC), in strengthening Ethiopia's wheat breeding programs and advancing food security. Such collaborations can reduce reliance on wheat imports, enhance agricultural resilience, and address food security challenges.

*[P26] Evidence of spatial divergence of *Blumeria graminis f. sp. tritici* pathotypes in Australia

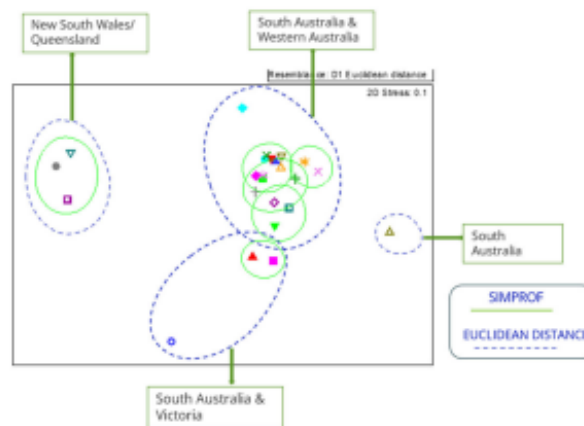
Ancy Kuthokathan Tony¹, Simon Ellwood^{1,2}, Huyen Phan^{1,2}, Daniel Mullan³, Fran Lopez Ruiz^{1,2}, Benjamin J. Saunders², and Kar-Chun Tan^{1,2}

¹Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Australia; ²School of Molecular and Life Sciences, Curtin University, Bentley, Australia ³InterGrain Pty Ltd, Bibra Lake, Australia

Correspondence to: ancy.kuthokat@postgrad.curtin.edu.au

Wheat powdery mildew (WPM) is caused by the obligate biotrophic fungus *Blumeria graminis* f.sp. *tritici* (*Bgt*). WPM contributes to major yield losses and economic damage to the Australian cereal industry. Current strategies to mitigate the impact of WPM include crop rotation, chemical control and genetic resistance. However, due to the common and continuous use of fungicides, fungicide-resistant WPM have been reported across Australia. This highlights the genetic resistance is an effective and sustainable solution to control WPM. There are no reports on the pathotype or population structure of Australian *Bgt*, nor the WPM resistance gene complements in Australian wheat varieties. In this study, *Bgt* isolates were collected from across Australia between 2020 and 2024. We assessed the level of virulence of these *Bgt* isolates using a diverse wheat panel consisting of varieties with defined WPM resistance (*R*) genes. Differences in aggressiveness level of *Bgt* isolates on the panel were observed. Non-metric multidimensional scaling (nMDS) of virulence data revealed evidence of multiple distinct *Bgt* pathotypes that are distributed across different wheat growing regions. Analysis of wheat variety resistance identified major *R* genes such as *Pm3a*, *Pm16*, *Pm4a*, *Pm4b* and *Pm2* that are effective against all tested *Bgt* isolates. Thus, we recommend strategically stacking and deploying these *R* genes into commercially grown wheat varieties to prolong genetic resistance and minimise the impact of WPM.

Fig: nMDS analysis identified multiple pathotypes in Australian WPM



***[P27] Differentiating actively replicating vs. non-replicating forms of Hop Latent Viroid**

Heather H Tso, Cameron Scott, Barbara M Wong, and Zamir K Punja

Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada, V5A 1S6 Correspondence to: zamir_punja@sfu.ca

Hop Latent Viroid (HLVd) is a threat to the cannabis (*Cannabis sativa*) industry, leading to economic losses from reduced yields. HLVd is circular RNA molecule 256 nucleotides long. It replicates through a rolling circle mechanism, producing linear concatemeric intermediates followed by mature circular forms. Accurate identification of HLVd replication status is essential

for understanding viroid epidemiology. It is unclear whether RT-PCR bands of ~250bp, ~500bp, and ~750bp observed in electrophoretic gels following extraction from infected tissues represent circular or linear RNA, or whether it corresponds to viroid titres. HLVd-infected samples were collected and total RNA was extracted. The RNA samples were treated with RNase R, an exoribonuclease that degrades linear RNA while not affecting circular RNA. Treated and untreated RNA samples were subjected to RT-PCR using HLVd-specific primers. Untreated samples displayed all three distinct bands. In treated samples, the ~500 bp and ~750bp bands were degraded, confirming these to be linear RNA, while the ~250bp band remained intact, consistent with the circular form. Seeds, resin, dried buds, and trichomes only showed the ~250bp band, suggesting the non-replicative form was present. In contrast, ~500bp and ~750bp bands were detected in roots, stems, and leaves, suggesting these tissues support active replication. qPCR results indicate that viroid titre is elevated in samples with the two higher molecular weight bands. These findings provide a molecular diagnostic framework for distinguishing replicating versus non-replicating HLVd. This information can provide a basis for assessing infection risk and targeting replication hotspots to improve viroid management in cannabis cultivation.

***[P28] Genomic analyses of a sexually recombining population of the wheat stem rust pathogen from Spain identifies putative avirulence loci**

Molly Veregge¹, Dolores Villegas², Carlos Cantero-Martinez³, Melissa Carter⁴, Douglas Luster⁴, Matthew Moscou⁵, Yue Jin⁵, Pablo Olivera Firpo¹

¹Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA; ²Sustainable Field Crops Program, Institute of Agrifood Research and Technology (IRTA), Lleida, Spain; ³Crop and Forest Science Department, University of Lleida, Agrotecnio CERCA Center, Lleida, Spain; ⁴USDA-ARS Foreign Disease-Weed Science Research Unit, Ft Detrick, MD, USA; ⁵USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN, USA

The wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici* (*Pgt*), has been responsible for historically devastating epidemics that resulted in complete wheat crop losses. In the 20th century, barberry eradication programs across Europe and the United States contributed to the control of this disease by removing the sexual stage of the pathogen's life cycle, limiting the occurrence of new virulent races. The resurgence of common barberry across Europe has resulted in unexpected outbreaks of wheat stem rust where it was only marginally detected for over 50 years. We characterized a *Pgt* population of sexual origin derived from 21 aecial and uredinial samples collected from native barberries and adjacent wheat fields in 2019 in Spain. This highly diverse population of 200 unique races exhibit novel and significant virulence combinations, including on multiple Ug99-effective genes, and on *Sr31*, a trait only seen in races of the Ug99 group. To identify underlying avirulence genes in *Pgt* via association studies, we generated Illumina sequencing data for 154 isolates segregating on 18 stem rust resistance genes. Genomic analyses revealed markedly high levels of genetic diversity, and we identified 14 novel alleles of cloned *Pgt* effectors to be functionally validated. Additionally, GWAS identified loci containing putative effector genes representing new targets for gene cloning and evaluation. Overall, the immense

phenotypic and genotypic diversity of this population is aiding in the discovery of relevant avirulence loci, contributing to the understanding of evolution of virulence of the stem rust pathogen.

***[P29] Bacterial leaf streak: Investigating the dynamics of pathogen virulence and host resistance**

Vinuri Weerasinghe^{*1}, Malini Jayawardana¹, Shaheen Bibi, and Dilantha Fernando¹

¹Department of Plant Science, University of Manitoba, Winnipeg, MB, R3T 2N2

Correspondence to; weerasi1@myumanitoba.ca

Bacterial Leaf Streak (BLS) is a worldwide distributed bacterial disease caused by *Xanthomonas translucens* (Xt). Its economic impact is significant on small grain cereal crops. BLS in barley is caused by *X. translucens* pv. *translucens* (Xtt) and *X. translucens* pv. *undulosa* (Xtu) whereas wheat is infected by the latter pathovar. The recent emergence of BLS in the Canadian prairies is concerning. Aiming to find resistant germplasm, we evaluated Canadian barley and wheat genotypes against a panel of BLS isolates representing Canada and the United States (US), under greenhouse conditions. The pathogen inocula were syringe-infiltrated to the secondary leaf, 14 days after seeding. The disease severity was rated using a 0-5 scale, five days post-inoculation. A few barley genotypes resistant for Xtt and/or Xtu were identified although BLS-resistance was not found in wheat genotypes tested so far. To understand the dynamics in pathogen virulence over time, we compared disease severity between historical and modern BLS isolates on barley using a two-way Analysis of Variance. The virulence of historical BLS isolates was not significantly different from modern isolates ($p < 0.05$). Phylogenetic analysis suggested potential genetic variation between Canadian and US populations of Xt. The pathosystem will be further explored for pathogen localization, transmission, and pathovar interactions. Moreover, field studies will be conducted for mapping adult plant resistance in spring wheat and winter wheat. The findings of this study will immensely contribute to minimize the potential impact of BLS on the wheat and barley industries in Canada.

***[P30] Monitoring the evolution of stripe rust in Canada over the past 40 years**

Bohan Wei^{1,2}, Ryan Gourlie¹, Rhodesia Celoy¹, Rodrigo Ortega Polo¹, Nathaniel Lim¹, Stephen E. Strelkov², and Reem Aboukhaddour¹

¹Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403 1 Avenue S, Lethbridge, AB, Canada, T1J 4B1; ²Department of Agriculture, Food and Nutritional Science, University of Alberta, 116 St & 85 Ave, Edmonton, AB, Canada, T6G 2R3

Correspondence to: reem.aboukhaddour@agr.gc.ca

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), threatens global food security. In Canada, the risk of stripe rust has increased since 2000 due to the emergence of new races with

broad virulence spectra, adapted to higher temperatures. However, since 2010, the *Pst* population in Canada has shifted further, with increased virulence overcoming additional, previously effective *Yr* genes. To understand this shift, we analyzed 31 assembled genomes generated from isolates collected across Canada from 1984 to 2023 and sequenced with short-reads. SNP calling and mating-type allele analyses were also conducted. Our results indicate a shift in the *Pst* population in Canada from the dominant PstS0 lineage before 2000 to a PstS1-related lineage after 2010. Additionally, two isolates collected around 2000 shared *Pst-b1-HD*, *Pst-b1*-HD*, and *Pst-b8-HD* alleles, identifying them as a South African lineage, which in addition to PstS1, may have contributed to the incursion of heat adapted races into North America. Several isolates exhibited recombinant patterns from various lineages, including the previously reported PstPr. Notably, we identified a novel lineage, PstC-2022, in two isolates from eastern Canada collected in 2022 and 2023. This may be a recombinant lineage between PstS1 and a foreign lineage from the Eurasian-East African Corridor. SNP analysis revealed distinct genetic clusters correlating with sampling periods and lineage designation rather than geographic origin, highlighting the pathogen's capacity for long-distance dispersal. Overall, this study underscores the value of phenotyping and genotyping for accurately tracking *Pst* virulence, evolution, and long-distance incursions.

[P31] Genotypic and phenotypic changes in *Fusarium graminearum* under adaptive evolution to azole selection pressures

Kelsey Wog, Aleeza Gerstein, Matthew Bakker

University of Manitoba, Department of Microbiology, Winnipeg MB, Canada

Correspondence to: Matthew.Bakker@umanitoba.ca

Fusarium graminearum is a major pathogen of wheat and barley, causing annual global agricultural losses exceeding one billion dollars. In Canada, azole fungicides (demethylation inhibitors) have historically effectively managed *F. graminearum* infections, but recent data suggests increasing resistance in populations. Azoles inhibit ergosterol biosynthesis by binding to the active site of Cyp51. Resistance to azoles is often driven by mutations in the *cyp51* gene that alter the enzyme binding site, lowering azole binding affinity. In this study, *F. graminearum* strain DAOMC 233423 was exposed to increasing concentrations of tebuconazole, prothioconazole, a combination of both fungicides, or a solvent carrier control (4 replicates each) until strain extinction in the fungicide treatments. One lineage evolved to tebuconazole acquired resistance to tebuconazole as well as cross-resistance to prothioconazole and the combination treatment and also displayed impaired spore production. Whole genome sequencing analyses identified a single base insertion in the *smt3* gene, resulting in a premature stop codon. A second lineage evolved to tebuconazole was significantly more pathogenic on wheat than the ancestral strain but displayed less tolerance to tebuconazole than the other 3 replicates evolved to tebuconazole. This strain had a missense mutation in the *fzo/ugo1/mgm1* gene (related to mitochondrial membranes). One lineage evolved to the combination treatment displayed more cellular branching, with a missense mutation in the *gt31* gene (glycosyltransferase). We are creating gene knockouts to investigate the function of these genes and determine whether they play a role in azole resistance.

***[P32] Impact of storage conditions on the viability and virulence of *Plasmodiophora brassicae* resting spores**

Chunxiao Yang¹, Andrea Botero-Ramirez², Sheau-Fang Hwang¹, and Stephen E. Strelkov¹

¹Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2P5; ²Department of Biological Sciences, MacEwan University, Edmonton, AB, Canada, T5J 4S2

Correspondence to: strelkov@ualberta.ca

Clubroot disease, caused by the soilborne pathogen *Plasmodiophora brassicae*, poses a significant threat to *Brassica* crop production worldwide. The resting spores produced by *P. brassicae*, which can survive in soil for up to 20 years, serve as the primary inoculum source, driving epidemics in susceptible hosts and contributing to resistance breakdown in resistant cultivars. Although spore viability declines significantly under field conditions following a 2-year rotation out of susceptible hosts, residual spores may persist at low levels for decades. However, the long-term effects of laboratory storage conditions on the viability and virulence of resting spores within clubroot galls remain poorly understood. This information is critical for maintaining viable and virulent *P. brassicae* collections to support research and resistance-screening efforts. This study investigates temporal changes in resting spore viability and virulence under different storage conditions. Field-collected clubroot galls were stored in darkness at room temperature, -20°C, and -80°C for periods ranging from 1 to 19 years. Preliminary assessments revealed that samples stored at room temperature retained the highest spore viability over time, followed by those stored at -20°C and -80°C. Ongoing research will assess the virulence of stored spores through bioassays on susceptible *Brassica* hosts to determine correlations between viability and infectivity. Findings from this study will enhance our understanding of *P. brassicae* spore longevity under various storage regimes, facilitating research aimed at developing improved clubroot management strategies and durable resistance.

***[P33] Analysis of Fusarium crown rot severity caused by the Canadian and Italian *Fusarium culmorum* isolates under varying soil moisture stresses**

Y.M.Shashika M. Yapa¹, James R. Tucker², W.G.Dilantha Fernando¹,

¹Department of Plant Science, Room 222 Agriculture Building, 66, Winnipeg, MB R3T 2N2, Canada; ²Agriculture and Agri-Food Canada, Brandon Research and Development Centre, Brandon, MB R7A 5Y3, Canada.

Correspondence to: dilantha.fernando@umanitoba.ca

Fusarium crown rot (FCR), caused by *Fusarium culmorum*, is a significant soilborne disease of wheat, affecting yield and food safety. While environmental factors like soil moisture influence disease development, the impact of isolate origin on pathogen aggressiveness remains understudied. Thus, this study aimed to assess whether abiotic stress, specifically moisture

stress, modulates the aggressiveness of *F. culmorum* isolates from contrasting regions on Durum wheat. Four *F. culmorum* isolates, two from Canada and two from Italy, were inoculated onto two-week-old seedlings. Plants were subjected to three watering regimes simulating varying moisture stresses such as daily (optimal), every two days (moderate stress), and every four days (severe stress). Disease severity was scored 15 days post-inoculation using a standardized 0–6 scale reflecting crown rot necrotic lesions. According to the results, there is no significant interaction was observed between isolate and moisture treatment. However, results showed a significant effect of isolate on disease severity ($p < 0.001$), with Canadian isolate 271D exhibiting the lowest severity. These findings suggest that short-term moisture stress may have a limited influence on FCR development regardless of its origin or the moisture content in the soil. This study highlights the importance of considering isolate-specific aggressiveness in breeding and disease management programs. As a next step, the effect of temperature on disease development and isolate aggressiveness will also be analyzed. Moreover, conducting separate experiments with Canadian and Italian wheat cultivars would offer valuable insights into host–pathogen interactions.

[P34] Exploring the diversity of Quebec wheat cultivar resistance genes to leaf rust (*Puccinia triticina*)

Tanya Copley¹, and Silvia Rosa¹

¹Centre de recherche sur les grains (CÉROM), inc., 740, chemin Trudeau, Saint-Mathieu-de-Beloeil, Québec, J3G 0E2

Correspondence to: tanya.copley@cerom.qc.ca

Wheat leaf rust (*Puccinia triticina* Eriks.) is the most commonly observed and distributed wheat rust in Québec. Recent annual rust race surveys indicated that the race profiles in Québec are more diverse than the populations from the Canadian Prairies. Several factors may explain the high genetic diversity of leaf rust races in Québec, including inoculum arriving from various regions of the USA, as well as selective breeding. This objective of this study is to examine the genetic diversity of leaf rust resistance genes in Québec spring and winter wheat cultivars. To do so, previously characterized *P. triticina* races from the 2023 and 2024 growing seasons were used for inoculation assays on a total of ten winter wheat and 28 spring wheat cultivars commonly used in agricultural fields. Disease reactions to the races were assessed two weeks post-inoculation according to the 1 to 4 scale defined by Long and Kolmer (1989). Reaction types 1 and 2 were considered resistant to the race, while reactions 3 and 4 were considered susceptible. Results to date suggest that Québec winter wheat cultivars are generally more susceptible to the tested races than spring wheat cultivars. Resistant gene diversity in Québec winter wheat cultivars also appears to be less diverse than in spring wheat cultivars, with similar reaction types in all winter wheat cultivars. The genetic diversity of leaf rust resistance genes in Québec spring wheat cultivars appears to be more diverse, with at least half of the tested cultivars being resistant to a given race.

***[P35] Investigating the Naturally Occurring Genetic Variability of *AvrLm2* and *AvrLm7* in *Leptosphaeria Maculans* Across Canadian Canola Fields**

Shaheen Bibi¹, Liang Zhao¹, Sean Walkowiak² and Dilantha Fernando¹

¹Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2; ²Canadian Grain Commission

Correspondence to: dilantha.fernando@umanitoba.ca

Blackleg, caused by *Leptosphaeria maculans*, remains a significant threat to canola production in Canada. While resistance (R) genes such as *Rlm3* initially provided effective control, the pathogen has rapidly evolved, rendering some resistance genes ineffective in commercial fields. In 2022, new canola cultivars containing *Rlm2* and *Rlm7* were introduced, offering renewed promise for disease management. This study was initiated to monitor and understand the genomic changes in *L. maculans* populations that could lead to the breakdown of these newly deployed resistance genes. Among thousands of isolates we had in our lab; we were able to identify a few isolates that had *avrLm2* and *avrLm7* from Canadian fields. These were isolated prior to the introduction of *Rlm2* and *Rlm7* carrying cultivars. To capture early evolutionary dynamics, pathogen isolates were collected from experimental plots and commercial farms where *Rlm2* and *Rlm7* containing cultivars were grown. High-quality genome sequencing was performed using Oxford Nanopore technology. Assemblies were generated using Flye and assessed for completeness with BUSCO and compleasm. Comparative genomic analyses were conducted against a reference *L. maculans* genome and across isolates collected over multiple years. SNP analyses were performed using dnadiff. Our findings reveal emerging genetic changes in avirulence genes associated with *Rlm2* and *Rlm7* interactions, including point mutations and gene disruptions. These results suggest early signs of adaptive evolution in the pathogen population. This research provides a foundational genomic dataset to inform resistance gene stewardship and offers valuable insights into the evolutionary potential of *L. maculans* under field conditions.

[P36] Understanding the pathogen diversity and potential management options for diseases caused by *Neopestalotiopsis* spp., an emerging threat of Canadian berry production

Rishi R. Burlakoti¹, Amy Novinscak¹, and Justin Renkema²

¹Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, 6747 Highway 7, Agassiz, BC, Canada, V0M 1A0; ²London Research and Development Centre, Agriculture and Agri-Food Canada – Vineland Campus, 4902 Victoria Ave N Vineland Station, ON Canada L0R 2E0

Correspondence to: rishi.burlakoti@agr.gc.ca

Neopestalotiopsis spp. are fungal pathogens that cause canker, shoot decay, fruit rot, leaf spot and blight, and crown rot in horticultural crops including berry fruit crops, guava, macadamia, avocado, rubber trees etc. In particular, the pathogen caused severe damage in strawberry plants in commercial fields in Florida in recent years, and the pathogen spread to several states in the USA and is in Ontario and Atlantic Canada. We also found two species of *Neopestalotiopsis*, infecting lingonberry plants (*Vaccinium vitis-idaea* L.) in Canada. A detailed

characterization of the pathogen, its spread in Canada, and its variability in pathogenicity and virulence will be discussed. There are no fungicides registered in Canada for *Neopestalotiopsis* spp. in berry crops. We will discuss the potential fungicides and other non-chemical management options including transplant steam treatment to control the disease in berry crops.

***[P37] Evaluation of host resistance and antagonistic bacteria for the management of Verticillium stripe in canola**

Jorge Cordero-Elvia, Karla Flores Carrera, Sheau-Fang Hwang, and Stephen E. Strelkov

Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB T6G2P5, Canada

Correspondence to: jcordero@ualberta.ca

The emergence of Verticillium stripe, caused by *Verticillium longisporum*, represents a significant challenge to canola (*Brassica napus*) production, as no resistant cultivars are currently available. Biological control using root-associated bacteria offers a promising alternative strategy for disease management by inhibiting pathogen development and potentially enhancing plant defense responses. This study aimed to identify canola breeding lines and rhizosphere-derived bacterial strains capable of suppressing *V. longisporum*. A total of 25 canola lines were evaluated under controlled greenhouse conditions for resistance to isolate VL 43, with disease progression quantified using the normalized Area Under the Disease Progress Curve (AUDPC_norm). Two lines exhibited significantly lower AUDPC_norm values than the susceptible check *B. napus* 'Westar' and were statistically comparable to the resistant check *B. rapa* ECD 05, indicating moderate resistance. In addition, 378 bacterial isolates from the canola rhizosphere were screened for antagonistic activity against *V. longisporum* using dual culture assays. Fifty-one strains demonstrated inhibitory effects, with the strongest antagonism observed in strains of *Agrobacterium arsenijevicei*, *Bacillus wiedmannii*, *Paenibacillus polymyxa*, *Paenibacillus terrae*, *Peribacillus frigitolerans*, *Pseudomonas silesiensis*, and *Streptomyces xiangtanensis*. Ongoing greenhouse trials are evaluating the biocontrol efficacy of the ten most inhibitory strains, both individually and as synthetic microbial communities. These findings highlight the potential for integrating host resistance with biocontrol strategies to manage Verticillium stripe in canola, offering a sustainable approach to improving canola production.

***[P38] Evaluation of Stripe Rust Symptoms in the Winter Wheat Population '25R56' X 'E1007R', and Selected Checks in Ontario, Canada**

Tamburic-Ilincic L., Serajazari, M., Navabi A., and Pourkhorshid, Z.

University of Guelph, Department of Plant Agriculture, Guelph, Ontario.

Correspondence to: ltamburi@uoguelph.ca

Wheat rust diseases (leaf, stem, stripe) cause significant economic losses worldwide. Stripe or yellow rust (YR), caused by *Puccinia striiformis* sp. *tritici* (Pst), can lead to yield losses exceeding

30%. In Ontario, the initial outbreak of YR was documented in 2016, with subsequent occurrences happening sporadically. Host resistance and fungicide applications are effective strategies to control YR. The objective of this study was to evaluate YR symptoms and agronomic traits in a winter wheat doubled haploid (DH) population, derived from the cross between '25R56' (resistant, rated 1) and 'E1007R' (susceptible, rated 7), based on a 0–9 scoring scale, along with several check varieties, at the Ridgeway and Elora Research Stations in Ontario, during 2015 and 2016. The selected control varieties were additionally assessed in subsequent years, including 2024, when the disease was reported in multiple counties. In addition, the GreenSeeker Handheld Crop Sensor was used to record the normalized difference vegetation index (NDVI), with 0-0.99 values, six times per growing season. Transgressive segregants were observed for all measured traits, including plant height, heading date, test weight, yield, NDVI, and YR. The interactions between genotype, year, and location were significant for yield and sum-NDVI. Average YR showed a significant, but weak negative correlation with yield, plant height, and test weight. The strong positive correlation was observed between yield and sum-NDVI ($r=0.87$) and yield and test weight ($r=0.65$). Wheat lines with higher NDVI values were healthier and yielded more. The results have significant implications for wheat breeding and selection.

***[P39] Haplotype-phased genomes reveal the evolutionary history of race groups TKTF,**

TTRTF and avirulence gene variants associated with severe wheat stem rust epidemics

Rebecca E Spanner¹, Eva C Henningsen², Feng Li¹, Oadi Matny¹, David P. Hodson³, Nino Virzi⁴, Kim-Phuong Nguyen⁵, Jibril Lubega⁶, Kostya Kanyuka⁶, Matthew Moscou⁵, Zacharias A. Pretorius⁷, Willem Boshoff⁷, Jana Sperschneider⁸, Peter Dodds⁸, Brian Steffenson¹, Melania Figueroa⁸

¹University of Minnesota, St. Paul, MN, U.S.A. 55108; ²Research School of Biology, The Australian National University, Canberra, ACT, Australia 2601; ³International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico 56237; ⁴Centro di Ricerca Cerealicoltura e Colture Industriali (CI), Acireale, CT, Italy 95024; ⁵Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A. 55108; ⁶NIAB, Cambridge, United Kingdom CB24 9NZ; ⁷University of the Free State, Bloemfontein, South Africa 9300; ⁸Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT, Australia 2601

Correspondence to: Melania Figueroa (melania.figueroa@csiro.au) and Peter N Dodds (peter.dodds@csiro.au)

A recent milestone in dikaryotic rust (*Puccinia*) genomics has been the development of haplotype-phased genomes. This has enabled tracking of entire nuclear haplotypes to reveal evidence of somatic hybridisation events leading to the emergence of novel race groups (e.g. TTKSK/Ug99). For their roles in recent severe wheat epidemics and virulence on widely deployed R genes, we performed PacBio HiFi and Hi-C sequencing for isolates ETH2013-1 and ITA2018-1. ETH2013-1 is of race group TKTF which dominated during the severe 2013-2014 epidemic on wheat cultivar Digalu after overcoming *SrTmp*. ITA2018-1 is of race TTRTF which overcame *Sr13b* and *Sr35* causing severe epidemics on durum wheat in Sicily, Italy during 2016-17. The phased nuclear genomes

revealed novel nuclear haplotypes G and H in ETH2013-1 and haplotypes I and J in ITA2018-1. The TKTF race group is genetically diverse: the first described TKTF “type” strain 13ETH18-1 (Olivera *et al.* 2015, *Ecol. Epidemiol.* 105:917-928) is in Clade IV-A and is genetically distinct from ETH2013-1 (Clade IV-B). *K*-mer containment analysis of Illumina reads revealed that German and Georgian isolates in Clade IV-C contain haplotype H of ETH2013-1, but not haplotype G, suggesting a historical nuclear exchange event. Novel variants for known avirulence effectors (*AvrSr13*, *AvrSr22*, *AvrSr27*, *AvrSr35* and *AvrSr50*) and candidates for additional avirulence effectors were mined and tested for recognition using functional assays. We have established novel allele nomenclature for an *AvrSr* gene atlas comprised of functionally characterized *AvrSr* variants which can be used to determine the virulence of surveyed isolates on deployed *Sr* genes.

Figure:

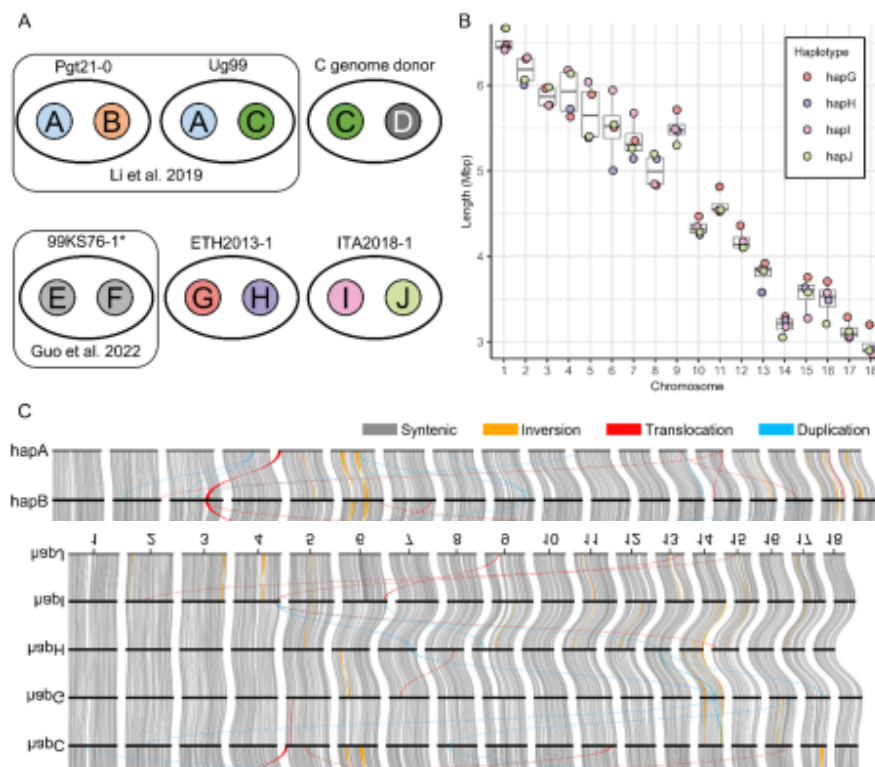


Figure 1. a) Nuclear haplotype designations for *P. graminis* f. sp. *tritici* isolates. Asterisk indicates that available reference haplotypes are not nuclear phased. **b)** Boxplots of chromosome lengths for haplotypes from ETH2013-1 and ITA2018-1. Box boundaries represent the first and third quartile and the center line represents the median length. Colors represent nuclear haplotypes. **c)** Synteny plots showing syntenic regions and incidences of inversion, translocation and duplication after pairwise alignments between haplotypes.

***[P40] Virulence diversity and molecular characterization of *Verticillium longisporum* isolates recovered from canola in Manitoba**

L. WU¹, Y. WANG¹, A. ABDELMAGID², F. DAAEF³, G. CHONGO⁴, S.E. STRELKOV¹, R. FREDUAGYEMAN¹ AND S.F. HWANG¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; ²Agriculture and Agri-Food Canada, Morden, MB R6M 1Y5; ³University of Manitoba, Winnipeg, MB R3T 2N2; ⁴BASF Canada Inc., Saskatoon, SK S7K 3J9
Correspondence to: sh20@ualberta.ca

Verticillium stripe of canola (*Brassica napus*) is caused by *Verticillium longisporum*. The A1/D1 lineage group of this pathogen was previously identified as dominant in Manitoba, where the disease is most widespread. In 2024, stem tissues exhibiting typical Verticillium stripe symptoms were collected during a field survey of 68 canola crops in Manitoba. Fungal isolates were recovered from these samples and putatively identified as *V. longisporum* based on conidiophore structure, conidial shape, and the presence of microsclerotia. Single-spore isolates (SSIs) were obtained from the putative *V. longisporum* cultures, and species identification was confirmed by PCR using two species-specific primer pairs (VlspF1/VlspR4 and VerniF2/VernR3), a multiplex primer set (D1f, AlfD1r, A1f, A1r, Df, and Dr), and the ITS4/ITS5 primers targeting the internal transcribed spacer region. Amplicons were subjected to Sanger sequencing and BLAST analysis. The pathogenicity of the SSIs was evaluated under greenhouse conditions. Verticillium stripe symptoms were observed in 37 of the 68 surveyed fields, with 60 putative *V. longisporum* colonies recovered. From these, a total of 117 SSIs were obtained. Molecular testing confirmed all isolates as *V. longisporum*, belonging to the A1/D1 lineage group. Pathogenicity tests indicated considerable variation among SSIs. Based on least significant difference (LSD) analysis, six avirulent and 72 highly virulent isolates were identified, with further testing underway. These findings provide important insights into the distribution, identity, and virulence diversity of *V. longisporum* populations in Manitoba, contributing to improved disease management and resistance breeding strategies in canola.

[P41] One Host, Many Rusts: An Overview of *Puccinia* Species with Aecial Hosts on *Berberis* in North America

Mehrdad Abbasi¹ and Gurcharn Singh Brar^{1,2}

¹Faculty of Land and Food Systems, The University of British Columbia (UBC), Vancouver, BC, Canada, V6T 1Z4; ²Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada, T6G 1C9 Correspondence to: puccinia@gmail.com

Infecting two phylogenetically unrelated host plants is one of the characteristic features of heteromacrocytic species of rust fungi, which is also called heteroecism. Heteroecism is quite common among graminicolous rust fungi. In this case, uredinia and telia are produced on members of the Poaceae, while spermogonia and aecia of the same species are produced on different members of monocotyledonous or dicotyledonous angiosperms. One of the well-known aecial hosts among heteromacrocytic rusts of cereals and grasses is members of the genus *Berberis* (including *Mahonia*) from Berberidaceae. So far, worldwide, in addition to *Cumminsia mirabilissima* and *Puccinia oxalidis*, aecia of ten graminicolous *Puccinia* species have been reported on *Berberis* spp. Records from North America indicate the presence of *C. mirabilissima*, *P. oxalidis*, *P. graminis*, *P. pseudosrtiiformis*, *P. striiformoides*, *P. poae-nemoralis*, *P. arrhenatheri*, *P. pygmaea* and *P. montanensis* with either a complete life cycle or a partial cycle characterized by

the production of uredinia and telia. Here, we present an overview of all rusts with aecia on *Berberis* sensu lato in North America, including those with confirmed presence and those known only from their telial state but with potential for the presence of aecia. We also include the host range and distribution of the aecial state of these rusts based on literature and herbarium records. A pictorial identification key is presented to distinguish the above rusts based solely on aecial characteristics observed on *Berberis*. The results of this study may serve as a practical reference for field guides or lab diagnostic tools.

[P42] Development of Multiple Rust-Resistant Durum Wheat Genotypes and Identification of Resistance Sources Using Genome-Wide Association Study (GWAS)

Muamar Al-Jaboobi¹, Seid Ahmed Kemal¹, Hafssa Kabbaj¹, Meryem Zaim¹, Filippo M Bassi¹

¹International Center for Agricultural Research in the Dry Areas (ICARDA)- Agdal Rabat 10080- Morocco.

Corresponding author*: Muamar Al-Jaboobi

Email: M.AJaboobi@cgiar.org

Durum wheat (*Triticum turgidum* ssp. *durum*) is an important major crop for home consumption and raw materials for food industries in many countries. The productivity and quality of durum wheat are affected by rust diseases which are an emerging challenge in dry areas where durum wheat is a major cereal crop. The aim of this work was to identify durum wheat genotypes with multiple resistance to yellow rust (YR), leaf rust (LR) and stem rust (SR). Forty-eight genotypes from ICARDA durum wheat breeding program were evaluated for YR, LR, and SR resistance at the seedling and adult plant stages. The seedlings were inoculated with a mixture of virulent pathogen populations collected from infected durum wheat plants in Morocco. These genotypes were also evaluated at the disease hot spots of Sidi Alall Tazi research station as well as in Guisch in Morocco. The phenotyping results revealed variation in resistance levels across the three rust diseases among genotypes at both the seedling and adult plant stages with the highest percentage of resistant genotypes observed for YR (81%), followed by LR (64%) and SR (54%). Seven genotypes demonstrated multiple resistance reactions to both leaf rust (LR) and stem rust (SR) at both the seedling and adult plant stages. Furthermore, a genome-wide association study (GWAS) identified significant SNP markers associated with resistance to LR, SR, and YR at the seedling stage, as well as to LR and SR at the adult stage. These evaluations indicate the potential for developing germplasm with multiple resistance to foliar rust diseases.

***[P43] Advances in Sexual Reproduction Related to Origin of New Races and Occurrence of Wheat Stripe Rust and Barberry Treatment for Managing the Disease in China**

Jie Zhao, Zhiming Du, Gensheng Zhang, Zejian Li, Maxinzhi Liu, Xinyao Ma, Mudi Sun, Bingbing Zhang, Zhensheng Kang

State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China

Correspondence to: jiezhao@nwafu.edu.cn

Historically, emergence of new races is responsible for the breakdown of genetic resistance of major-cultivated wheat cultivars in China. This concern has been focusing on the origin of new races. Recently, we observed that nearly fifty barberry (*Berberis*) species are widely distributed and commonly rusted in northwestern and southwestern regions in spring, and almost all are alternate hosts for the stripe rust. It is quite common that barberry bushes grow nearby wheat fields. Under natural conditions, sexual cycle of the fungus can occur regularly in northwestern region (a hotspot for the fungus) in spring, even in southwestern region in autumn. Importantly, aeciospore produced on susceptible barberry as inoculum (including new and known races) after infection spread to wheat fields to cause stripe rust in northwestern region in spring. Experiments we conducted uncovered that new virulent races of the stripe rust can be generated by sexual recombination including selfing, hybridization between races, and between *formae speciales* (f. spp.). Compared to mutation and somatic recombination, sexual recombination is an important way for the origin of new races, being used to clarify virulence variability and high genetic diversity of the stripe rust. Field investigations and indoor experiments showed that teliospore inoculum for sexual cycle mostly come from infected wheat plants, diseased wheat straws and debris, and grass hosts. At early pycnial stage, barberry bushes with spraying fungicides can terminate sexual development, significantly reducing rate of new races and aeciospore inoculum releasing from barberry to wheat and effectively managing stripe rust.

[P44] Early wheat rust disease detection and monitoring using very high-resolution satellite imagery in Ethiopia

Gerald Blasch^{1,5}, Ashenafi Gemechu², Tamirat Negash³, Tadesse Anberbir⁴, Girma Mamo⁴, Yoseph Alemayehu⁵, Francelino A. Rodrigues Jr⁶, David P. Hodson⁷

¹CIMMYT – Mexico, Texcoco, Mexico; ²Debre Zeit Agricultural Research Center (DZARC), Debre Zeit, Ethiopia; ³Kulumsa Agricultural Research Center (KARC), Asella, Ethiopia; ⁴Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia; ⁵CIMMYT – Ethiopia, Addis Ababa, Ethiopia; ⁶Lincoln Agritech Ltd, Lincoln University, Lincoln, New Zealand; ⁷CIMMYT – Nepal, Kathmandu, Nepal

Correspondence to: g.blasch@cgiar.org

Wheat rusts, particularly stripe and stem rust, pose a significant threat to Ethiopia's food security. In 2010, a stripe rust epidemic affected 600,000 ha, causing a loss of \$250 million. In 2014, a stem rust epidemic affected 30,000 ha, causing ~100% crop loss. Early detection and control are crucial. Very high-resolution satellite (VHRS) imagery offers potential for crop disease early warning systems, allowing for new detection methods at early growth stages, potentially revolutionizing early warning and response systems. The capability of multispectral VHRS systems was assessed as a rapid, early disease detection tool for wheat rusts. On-station experiments with varying irrigation and fungicide management were conducted in Ethiopia at the Kulumsa Research Center in 2020 and Debre Zeit Research Center in 2023. Wheat varieties with differing rust resistance were planted, and VHRS imagery was acquired over the experimental sites, coordinated with in-situ

visual disease inspections by pathologists. Our findings provide valuable insight into the capabilities of multispectral VHRS sensors for detecting, monitoring, and phenotyping the wheat rust disease at the plot level in general, and across healthy, mild, moderate, severe, and fully damaged disease situations, covering vegetative and reproductive growth stages. This research was the first time that satellite-based detection methods have been tested for stem rust. As wheat stem rust is now re-emerging as a disease of concern in Europe (and other regions), after an absence of over six decades, detection methods tested in Ethiopia may in the future find application and utility in other regions.

[P45] Gene stacking for enhanced durability of leaf rust resistance in the Canadian wheat variety Carberry

Firdissa E. Bokore¹, Colin W. Hiebert², Richard D. Cuthbert¹, Ron E. Knox^{1§}, Curtis J. Pozniak¹, Ron M. DePauw^{1§}, Yuefeng Ruan¹, Samia Berraies¹, and Brent D. McCallum^{2*}

¹Agriculture and Agri-Food Canada, Swift Current Research and Development Centre, 1 Airport Road, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada; ²Agriculture and Agri-Food Canada, Morden Research and Development Centre, 101 Route 100, Morden, MB R6M 1Y5, Canada; ³Department of Plant Sciences and Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

[§]retired.

Correspondence to: firdissa.bokore@agr.gc.ca; brent.mccallum@agr.gc.ca

Leaf rust (*Puccinia triticina* Eriks.) is an ongoing threat to wheat (*Triticum aestivum* L.) productivity. The spring wheat variety Carberry has exhibited a high level of resistance to leaf rust in Canada since its release in 2009. Understanding the genetic basis of resistance in Carberry is essential to the duplication of such resistance in future cultivars. Multi-year field nurseries for adult plant leaf rust evaluations were conducted at AAFC's Swift Current and Morden Research and Development Centres, along with greenhouse seedling leaf rust assessments of a doubled haploid population derived from a cross of Carberry with the susceptible variety Thatcher. Molecular mapping identified nine QTL/genes in Carberry associated with resistance on chromosomes 1B, 2B (2 QTL), 2D, 4A, 4B, 5A, 5B, and 7D. Six of these coincide with the genetic positions of known adult plant resistance genes (*Lr34*, *Lr46*, *Lr13*) and seedling resistance genes (*Lr16*, *Lr2a*, *Lr23*). Additive gene interactions, particularly between *Lr34*, *Lr46*, *Lr16*, and *Lr2a*, contribute to Carberry's durable resistance. Despite the evolution of virulent *P. triticina* strains, Carberry's resistance remains effective. Developed through doubled haploidy combined with conventional breeding selection strategies, Carberry demonstrates the value of irrigated, artificially rust-inoculated epiphytotic nurseries in breeding for resistance, complementing modern molecular breeding techniques.

***[P46] Fine mapping of the *Rpg7* barley stem rust resistance gene**

Matthew Brooke^{1,2}, Shaun James Clare¹, Arjun Upadhaya^{1,3}, Endeshaw Tadessee¹, Brooklyn Leonardo-Baily¹, Brian Steffenson⁴, and Robert Brueggeman¹

¹Department of Crop and Soil Sciences, Washington State University, Pullman, WA, 99161 USA;

²Department of Crop and Soil Sciences, Oregon State University, Corvallis, OR, 97330 USA;

³Department of Plant Pathology, Washington State University, Pullman, WA, 99161 USA;

⁴Department of Plant Pathology, University of Minnesota, St. Paul, MN, 55108 USA

Correspondence to: bob.brueggeman@wsu.edu

Virulence characterization of a stem rust (*Puccinia graminis* f. sp. *tritici*; *Pgt*) population from the Pacific Northwest revealed the most virulent *Pgt* population reported on barley. Several PNW isolates, including the aggressive Lsp21, exhibited virulence on barley stem rust *R*-genes *Rpg1*, *Rpg2*, *Rpg3*, *rpg4*, *Rpg5*, and *rpg8*. A wild barley (*Hordeum vulgare* ssp. *spontaneum*) diversity panel was screened against Lsp21, identifying accessions WBDC-94 and WBDC-238 as highly resistant. Both lines were previously shown to carry *Rpg7*, mapped to a ~10 Mbp region on chromosome 3H in the Morex v3 assembly. To fine-map *Rpg7*, a biparental mapping population was developed from cv. Morex (susceptible) × WBDC-94 (resistant). Phenotyping of 649 F₂ individuals with Lsp21 showed a 3:1 resistant-to-susceptible segregation ratio, consistent with a single dominant gene. Genotyping 436 F₂ individuals using an amplicon sequencing panel targeting 43 SNP markers across the *Rpg7* region identified 43 recombinants. Twenty-six recombinants with crossovers within or flanking the interval were advanced to the F_{2:3} generation and genotyped with seven PACE markers and an additional amplicon marker to further saturate the region. A high-resolution genetic map delimited *Rpg7* to a ~149 kb interval containing four high-confidence genes: an RPM1-like nucleotide-binding leucine-rich repeat (NLR), two FBD-associated F-box genes, and a Glutathione S-transferase T3-like gene. Two genes within the region contained polymorphism within their primary coding sequences that could explain resistance -vs susceptibility, including the NLR and one of the FBD-associated F-box genes. Based on predicted function and allele analysis the NLR is the top candidate *Rpg7* gene.

[P47] Population structure of yellow rust (*Puccinia striiformis* f. sp. *tritici*) in Poland in 2024

Piotr Słowacki, Urszula Piechota, Magdalena Radecka-Janusik, Dominika Piaskowska, and Paweł Czembor

Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland

Correspondence to: p.czembor@ihar.edu.pl

Yellow rust caused by the fungus *Puccinia striiformis* f. sp. *tritici* (Pst) is considered one of the most important pathogens of wheat. Understanding the genetic makeup of the pathogen population is essential for developing resistant varieties and promoting sustainable agricultural practices. In this study 44 single uredinium *Pst* isolates derived from both spring and winter wheat and triticale from six localizations in Poland in year 2024 were investigated. Analysis of the isolates was carried out using two molecular methods: analysis of SSR profiles for 19 loci and MARPLE (Mobile and Real-time Plant disease). In addition, selected isolates were phenotyped using a differential test. The SSR method is robust, easy to implement, and utilizes existing laboratory equipment to

characterize isolate races. MARPLE provides more detailed data on isolates but requires greater resources, including reagents, computing power, and data storage. Phenotyping is inexpensive regarding materials but is labor-intensive, time-consuming, and requires sufficient rust spores for infection assays. The analysis indicates that genetic group PstS10 (including Warrior(-) race) is dominant on both wheat and triticale. Specifically, group PstS10 had the largest number of isolates, with 20 isolates from wheat and 8 isolates from triticale, clearly highlighting its predominance. Additionally, wheat exhibited slightly greater genetic diversity, with isolates belonging to genetic groups: PstS7 (6 isolates), PstS8 (1), PstS10 (20), and PstS13 (1). In contrast, triticale had isolates identified only from genetic groups: PstS8 (1 isolate), PstS10 (8), and PstS13 (7).

[P48] Copper Stress Reshape Viral and Associated Microbial Communities and Impair Root Development and Architecture in Organic Soil

Azza Larafa^{1,2} and Mamadou L. Fall¹

¹Saint-Jean-sur-Richelieu Research and Development Centre, Agriculture and Agri-Food Canada, 430 Boulevard Gouin, Saint-Jean-sur-Richelieu, QC J3B 3E6, Canada; ²Department of Biology Science, Université de Montréal, 1375 Avenue Thérèse-Lavoie-Roux Montréal, Québec H2V 0B3, Canada

Correspondence to: [mamadolamine.fall@agr.gc.ca](mailto:mamadoulamine.fall@agr.gc.ca)

Organic soils are critical for sustainable agriculture and climate regulation, yet they are rapidly degrading under anthropogenic pressures. Copper is one very interesting example of a compound which plays multiples key roles for plant and microorganisms. Moderate levels of copper have beneficial effects in agricultural soils, especially as a component of fungicides and pesticides, which help control crop pathogens. However, repeated applications of copper-based agrochemicals can lead to the accumulation of excessive copper in soils, pushing it to toxic thresholds that severely impact microbial communities. This study investigates how copper contamination compared to soil sterilization alter the diversity and function of viral and microbial communities in organic soils and the consequences for root system architecture. Using high-throughput sequencing of virus-like particles and 16S rRNA amplicons, we analyzed community dynamics across untreated, copper-treated, and autoclaved soils over a lettuce growth cycle. We found that copper moderately reduced microbial and viral diversity, leading to delayed yet partial community recovery and root development. In contrast, autoclaving caused a severe collapse in biotic diversity, preventing long-term recolonization and reducing root complexity. In untreated soils, robust microbial-viral networks supported diverse, resilient communities and promoted fractal root growth. Environmental correlations highlighted pH and copper concentration as major drivers of biotic structure and function. These findings underscore the critical role of soil viruses, often overlooked, in shaping ecosystem resilience and provide new insights for organic soil health under environmental stress.

[P49] Molecular detection and geographic distribution of 3ANX-Producing *Fusarium graminearum* in the Maritime Provinces of Canada

Emily Johnstone¹, Abbey Saunders¹, Justin Renaud², and Adam Foster¹

¹Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, Charlottetown, PE, Canada; ²London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada Correspondence to: adam.foster2@agr.gc.ca

Fusarium graminearum, the primary agent of Fusarium head blight (FHB) in cereals, produces trichothecene mycotoxins that compromise grain safety. An emerging chemotype producing acetylated NX toxins presents new risks to cereal production, particularly in regions where its presence has not been previously confirmed. Recent development of qPCR assays targeting Tri1 gene polymorphisms specific to 3ANX, including two SYBR Green assays and one locked nucleic acid (LNA) probe assay, demonstrated high specificity and sensitivity. These assays were validated both in planta and in soil, enabling rapid and reliable detection of 3ANX DNA in infected material. In a 2021 survey of barley fields in New Brunswick, 20 *F. graminearum* isolates exhibiting the characteristic Tri1-NX RFLP pattern via Apol digestion were identified. All isolates with this pattern also tested positive using the 3ANX-specific qPCR assays, confirming that both diagnostic methods target the same Tri1 polymorphism. LC-MS/MS confirmed 3ANX toxin production in 19 of the isolates, and greenhouse pathogenicity assays showed that these isolates were virulent on barley, with NX toxins detected in infected spikes. This study represents the first confirmed report of 3ANX-producing *F. graminearum* in barley in New Brunswick and extends the known distribution of this chemotype throughout the Maritime Provinces of Canada. Continued surveillance using molecular and analytical tools is critical to understanding the spread and potential impact of this novel chemotype on cereal production and food safety in Atlantic Canada.

[P50] Verticillium stripe severity and yield impact in early- vs. late-maturing canola cultivars

L. WU¹, Y. WANG¹, L. GAO¹, S.E. STRELKOV¹, R. FREDUA-AGYEMAN¹ AND S.F. HWANG¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada Correspondence: sh20@ualberta.ca

Verticillium stripe of canola (*Brassica napus*), caused by *Verticillium longisporum*, is increasingly prevalent across western Canada. To date, only partial disease resistance has been reported. To assess the impact of maturity on Verticillium stripe development, six commercial canola cultivars with varying maturity times were evaluated in greenhouse experiments. The cultivars ‘P612L’, ‘CP22T1C’, and ‘DK902TF’ were early-maturing, while ‘BY 6217TF’, ‘P510G’, and ‘B4015’ were mid- to late-maturing. The old cultivars ‘Westar’ and ‘Topas’ were also included in all experiments and served as susceptible and partially resistant controls, respectively. Disease severity was assessed at multiple time points from the initial appearance of microsclerotia until plant senescence and was used to calculate the area under the disease progress curve (AUDPC). Plant height, survival count, and yield were recorded at full senescence. Least significant difference (LSD) analysis revealed that, under non-inoculated conditions, all six commercial cultivars produced higher and comparable yields relative to ‘Westar’ and ‘Topas’. However, in the inoculated treatments, significant yield losses were observed across all commercial cultivars except ‘B4015’, previously reported to carry resistance. Additionally, a reduction in plant survival

count was detected in all genotypes except 'B4015', while a reduction in height was only observed in 'Westar'. The AUDPC indicated that early-maturing cultivars, along with 'Westar', exhibited high to moderate disease progression. In contrast, mid- to late-maturing cultivars and 'Topas' displayed slower disease progression and reduced final disease severity. These findings suggest that early-maturing canola cultivars show earlier onset and greater severity of *Verticillium* stripe than later-maturing varieties.

[P51] Fungi associated with wild simulated ginseng leaves in central Canada

Abdurraouf Abaya¹, Melanie Kalischuk¹, and Paul H. Goodwin²

¹Department of Plant Agriculture, University of Guelph, 50 Stone Road, Guelph, ON, Canada, N1G 2W3; ²School of Environmental Science, University of Guelph, 50 Stone Road, Guelph, ON, Canada, N1G 2W3

Correspondence to: mkalisch@uoguelph.ca

Wild simulated ginseng (WSG) is the production of ginseng by planting within a natural forest. American ginseng (*Panax quinquefolius* L.) is a protected plant in its forest-dominated native habitat, but there is concern that WSG may result in undesirable outcomes, such as spreading ginseng diseases in its natural habitat. To determine the potential for WSG production in central Canada, a commercial WSG forest was evaluated for light dynamics, site characteristics, ginseng age and density, foliar symptoms and foliar fungal diversity. The forest stand was 60% beech, 39% sugar maple, and 1% ironwood, which had been managed for maple sugar production. WSG was found in the understory mainly located in 0.1-hectare clusters, ranging from 7,000 – 33,800 plants per hectare with the age of the ginseng plants from 2 to 8 years old. Ginseng clusters most frequently occurred at mid-slope meso positions with 8-35% slope and a southeast aspect. Leaves with foliar symptoms resembling diseases such as *Alternaria* leaf blight and insect damage were common with 5 to 71% showing damage by late August. Internal transcribed spacer (ITS) sequencing of randomly selected leaves revealed that the foliar microbiome diversity was relatively high with typical epiphytes and endophytes of leaves such as *Cladosporium herbarum* and *Aureobasidium pullulans*, and ginseng pathogens such as *Alternaria panax*. WSG shows potential for an alternative means of ginseng production in Canada with benefits, such as a relatively high microbial diversity.

[P52] Molecular diagnostics of the wheat leaf spot complex using the β -tubulin 1 gene

Mohamed Hafez, Dianeys González-Peña Fundora, Ryan Gourlie, Mouldi Zid, Reem Aboukhaddour*

Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

*Correspondence to: reem.aboukhaddour@agr.gc.ca

The wheat leaf spot complex is a globally significant foliar disease caused by multiple fungal pathogens: *Pyrenophora tritici-repentis* (tan spot), *Parastagonospora nodorum* and *Parastagonospora pseudonodorum* (septoria nodorum blotch), *Zymoseptoria tritici* (septoria

tritici blotch), and *Bipolaris sorokiniana* (spot blotch). Accurate diagnosis is challenging due to overlapping symptoms and similar morphologies. Current molecular tools often lack specificity, fail to detect all pathogens, or are not validated against other wheat-associated fungi. Notably, no assay specifically targets *P. pseudonodorum*, leading to underestimation of its role in leaf spot disease. To overcome these limitations, we developed a diagnostic toolkit targeting the conserved single-copy β -tubulin 1 (*tub1*) gene. Species-specific primers were designed for multiplex PCR (mPCR) and TaqMan-based quantitative PCR (qPCR), enabling simultaneous, sensitive, and specific detection. The qPCR accurately quantified pathogen biomass with detection limits as low as 0.04 pg of fungal DNA. Additionally, PCR-RFLP using selected restriction enzymes allowed clear species differentiation based on unique cleavage patterns. The developed assays showed no cross-reactivity with non-targeted fungi, including barley pathogens like *Pyrenophora teres*, ensuring reliability in agricultural systems where host overlap occurs. This molecular toolkit offers rapid and reliable detection, quantification, and differentiation of wheat leaf spot pathogens, supporting effective disease monitoring and enhance breeding for resistance.

[P53] Survey and characterization of rust pathogens associated with huckleberry (*Vaccinium membranaceum*), soapberry (*Shepherdia canadensis*), and other wild hosts in Pacific Canada

Sarah Hambleton^{1*}, Rishi Burlakoti^{2*}, Mehdi Sharifi³, Amy Novinscak², Julie Carey¹, Jordan Fraser³,
¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, Canada, K1A 0C6; ²Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, 6747 Highway 7, Agassiz, BC, Canada, V0M 1A0; ³Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, British Columbia, V0H 1Z0, Canada

Correspondence to: sarah.hambleton@agr.gc.ca; rishi.burlakoti@agr.gc.ca

Black huckleberry or mountain huckleberry (*Vaccinium membranaceum*) and soapberry (*Shepherdia canadensis*) are ecologically, culturally, and nutritionally important wild berry plants in North America. There are very limited studies on diseases impacting these wild berry plants. Therefore, we conducted surveys in British Columbia (BC), Canada, at four sites during summer months between 2021 to 2024 to monitor diseases caused by rust fungi. Forty-two infected samples were collected, mainly from four host plants: huckleberry, soapberry, subalpine fir (*Abies lasiocarpa*), and single-flowered clintonia (*Clintonia uniflora*). DNA sequences for the nrDNA ITS and partial 28S regions were compared to data generated for reference specimens deposited in the Canadian National Mycological Herbarium (DAOM) and to publicly available GenBank sequences. Analyses identified *Puccinia mesomajalis* on clintonia and *Puccinia coronati-calamagrostidis* on soapberry. Both species have been recorded previously from BC but our sequences are the first for *P. mesomajalis*, which has no alternate host and produces telia only. There were two species on black huckleberry: *Naohidemyces vaccinii* causing Hemlock-Blueberry rust, and *Calyptospora* spp. causing Witches' Broom Rust, which was also collected on its alternate host *A. lasiocarpa*. Our analyses of ITS sequences for *N. vaccinii*, including the only one available in

GenBank, suggested two sub-taxa with host and geographic differences. Our *Calyptospora* sequences grouped closest to GenBank data for *C. ornamentalis*, but there were some differences matching our data for DAOM specimens identified as *Peridermium holwayi*. More taxonomic investigation is needed for both rusts causing diseases of huckleberry in the BC sites.

[P54] Performance of Emerging Cropping Systems on Fusarium Head Blight in Wheat

Maria Antonia Henriquez¹, Ramona Mohr², Parthasarathy Santhanam¹, Christian Willenborg³, William May⁴, Brian Beres⁵

¹Morden Research and Development Centre, Agriculture and Agri-Food Canada, Unit 101 Route 100, Morden, MB, R6M 1Y5, Canada; ²Brandon Research and Development Centre, Agriculture and Agri-Food Canada, 2701 Grand Valley Road, Brandon, MB, R7A 5Y3, Canada; ³College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada; ⁴Indian Head Research Farm, Agriculture and Agri-Food Canada, 1 Government Road, Indian Head, SK, S0G 2K0, Canada; ⁵Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, AB, T1J 4B1, Canada

Correspondence to: mariaantonia.henriquez@agr.gc.ca

Fusarium head blight (FHB) is one of the most significant diseases affecting wheat and other small grain cereals worldwide. The expansion of soybean and corn production in Western Canada may prove to be one of the largest changes in cropping systems in this region since the introduction of canola in the 1970's. *Fusarium* spp. are causing disease in several economically important crops in Manitoba including soybean and corn. Recent research has shown that *Fusarium* species known to cause disease in wheat can also be pathogenic on soybeans and survive on the stubble of crops such as canola and pea. This cross-pathogenicity may have significant implications for managing *Fusarium* pathogens. In this research, we assessed soybean- and corn rotating systems from 2018 to 2022 at four different sites in western Canada. The sites were located at Brandon (AAFC), Lethbridge (AAFC), Saskatoon (University of Saskatchewan) and Indian Head (AAFC). We evaluated three different types of crop rotations; 1) 2- year cropping systems (wheat-canola, soybean-corn), 2) 3-year cropping systems (soybean-wheat-canola, corn- wheat-canola, corn-soybean-wheat, corn-soybean-canola) and 3) 4-year cropping systems (corn-soybean-wheat- canola). We investigated the effects of crop frequency and crop sequence on the severity of fusarium head blight in wheat. In addition, we monitored the population dynamics of *Fusarium* spp., deoxynivalenol (DON) content in the harvested grains, and quantified *Fusarium graminearum* and *Fusarium poae* using a multiplex Droplet Digital PCR (ddPCR).

[P55] Emerging disease threat: Neopestalotiopsis Spp. Identified in PEI strawberry farms

Cameron Menzies, Shauna L. Barry, Adam McLean, Suqi Liu, and Shuanglong Huang*

Department of Agriculture, Government of Prince Edward Island, 11 Kent Street, Charlottetown, PE, Canada, C1A 7N8

*Correspondence to: slhuang@gov.pe.ca

Strawberry (*Fragaria x ananassa*) is an economically important cultivated fruit crop species with a global presence. Recently, a new aggressive fungal species in the *Neopestalotiopsis* genus (*Neopestalotiopsis* spp.) has been shown to cause leaf spot, fruit, crown and root rot, and plant death in severe cases, and emerged as a significant threat in most strawberry production regions including Canada. This aggressive pathogen been found in strawberry plants in the provinces of Ontario, Quebec, Nova Scotia, and Prince Edward Island (PEI) in Canada. To monitor this emerging disease, a survey was conducted from strawberry farms across PEI in the Fall of 2024. A total of twenty-nine samples were collected from ten strawberry farms on the island and subjected to standard fungal isolation and molecular characterization by sequencing the PCR amplicons of the beta-tubulin gene. Preliminary results showed that, six isolates originated from three farms were confirmed *Neopestalotiopsis* positive. Sequence analysis categorized these six amplicons into two groups, four isolates were identical to the aggressive species, and two other isolates showed polymorphic variations, with four single nucleotide polymorphism (SNP) and one double-nucleotide substitution. The aggressiveness of these two unique isolates is currently unknown, and further analysis is underway. In the meanwhile, more strawberry samples will be collected for diagnosis in 2025. Joining Ontario and other provinces, PEI is requesting the emergency use registration of Allegro® 500F fungicide (Syngenta) to counteract this *Neopestalotiopsis* spp. in strawberry. Collectively, these results and actions not only ring the alarm bells for this pathogen in Canadian strawberry industry, but more importantly urge more research to study *Neopestalotiopsis* spp. and how to manage it effectively under different farming practices and environmental conditions.

***[P56] A genomic approach provided answers to unexplained disease symptoms of blueberries in British Columbia**

Sachithrani Kannangara¹, Juan Rodriguez¹, Adam Gilewski¹, Gerda de Villiers², Megan Ellis², Peter Ellis², Eric Gerbrandt³, Jim Mattsson¹.

¹Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada; ²Phyto Diagnostics Company Ltd., 9381, Ardmore Drive, North Saanich, BC V8L 5G4, Canada; ³BC Blueberry Council, 275 32160 South Fraser Way, Abbotsford, BC V2T 1W5, Canada.

Correspondence to: jim_mattsson@sfu.ca

In British Columbia, there are currently two viruses that cause severe disease and impact on commercial production of blueberries. While the symptoms of disease are initially similar, plants infected with Blueberry Shock virus can recover, whereas susceptible plants infected with Scorch virus never recover and ultimately die. Shock- infected plants can be left in the field whereas Scorch-infected plants should ideally be removed to prevent further spread of the disease. In recent years, however, growers have increasingly received test results that are negative for both viruses, indicating that one or several new viruses have emerged and may explain the hitherto undiagnosed disease. We used large scale RNA sequencing to identify potential novel viruses in healthy as well as diseased plants. We found four luteoviruses, two recently identified in the USA

and two novel luteovirus/variants. The luteoviruses are, however, widespread in healthy blueberry plants and not associated with disease. Instead, we found many novel variants of the Scorch virus and the Shock virus. When PCR testing was adjusted to identify plants carrying these variants as positive, the frequency of plants that tested positive for either or both of Scorch and Shock viruses increased dramatically and the number of false negative plants fell from 22% to 1% of plants. We also found that improved PCR also detects Scorch and Shock viruses at levels below that of cost-efficient ELISA testing. Taken together, detection of Scorch and Shock viruses rather than novel viruses appear to be the main reason behind undiagnosed disease.

[P57] The Sentinel Crop Disease Surveillance Network – a novel device for pest management

Camila M. Zanella¹, John Caulfield^{1,2}, Amelia Hubbard¹, Huw Davis¹, Christopher Blandford³, Bruce Grieve³, and Lesley A. Boyd¹

¹Niab, Villa Road, Histon, Cambridge, UK, CB24 9NZ; ²Rothamsted Research, Harpenden, UK, AL5 2JQ; ³Manchester Institute of Biotechnology, 131 Princess Street, Manchester, UK, M1 7DN.

Correspondence to: camila.zanella@niab.com

Airborne pathogens infect cereal crops reducing the yield. While their impact can be mitigated using fungicides, these must be used responsibly and sustainably. Many factors are known to influence the ability of cereal rust fungal pathogens to infect plants, including environmental variables as light, humidity and temperature, the topography of the leaf surface, as well as plant volatiles organic compounds (VOC). Our findings indicated the quantity of light (light intensity × duration) to which wheat seedlings were exposed influences VOC profiles and fungal infection levels for *Puccinia striiformis* f.sp. *tritici* (Pst), the causal agent of yellow rust (YR). Our studies indicate that VOCs collected from wheat subjected to high light intensity significantly enhanced spore germination. The understanding of the VOC profiles which enhanced Pst germination allowed us to integrate VOCs into a sensor biofilm in a novel disease surveillance tool named “Sentinel” to enhance spore germination and facilitate spore viability identification through AI. Sentinel is an innovative device designed to detect airborne spores as they enter crop field to improve pest management practices. Field trials conducted in 2022/2023 demonstrated efficacy in monitoring real time YR in wheat. The data from the biofilm sensors drove the timing of fungicide applications, resulting in reduced disease incidence in comparison to standard farmer fungicide applications. A second YR field trials are being conducted this field season (2024/2025). The data from each sensor can provide to the farmers an early warning of the presence and viability of the pathogen to drive the fungicide application regime.

[P58] Identification of the causal pathogen of an emerging disease on huckleberries:

Podosphaera myrtilina

Miao Liu¹, Rishi Burlakoti², Mehdi Sharifi³, Parivah Shoukouhi¹, Jordan Fraser³, Amy Novinscak², and Ziad El Hassan¹

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Ave, Ottawa, ON, Canada, K1A 0C6; ²Agassiz Research and Development Centre, Agriculture and Agri-

Food Canada, Agassiz, BC, Canada, V0M 1A0; ³ Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, BC V0H 1Z0 Canada

Correspondence to: miaomindy.liu@agr.gc.ca; rishi.burlakoti@agr.gc.ca

Black huckleberry (*Vaccinium membranaceum* Douglas ex Torr.) has long been part of the traditional diet of Indigenous Peoples in North America for millennia. Habitat disturbance, climate change, and disease spillover from surrounding ecosystems have caused significant mortality and diminished productivity in natural huckleberry patches. To assess the impact of biotic stresses on black huckleberry plant health and fruit harvest, comprehensive disease surveys were performed in 2023 and 2024. Among several observed disease symptoms, the incidence of purplish discoloration of the leaves was severe in huckleberry patches. The symptomatic leaves were collected to diagnose the disease and its causal agents. Morphological examination showed that the infection signs, along with the distinct characteristics of mycelia and chasmothecia (sexual structure) were consistent with *Podosphaera myrtillina* Kunze., a common pathogen of *V. myrtillus*, *V. oxycoccos*, *V. uliginosum*, *V. vitis-idaea* and *V. xintermedium* in Europe (GBIF, accessed on 2025-03-13), although it was rarely reported in Canada. Searching the major online host-pathogen databases, i.e. USDA Fungal-Host, AAFC Host-Pathogen (internal) and GBIF, collectively and respectively, revealed only one record of *P. myrtillina* on *V. membranaceum* and another on *Arctous* (= *Arctostaphylos*) *alpina* in the disease checklists by Amona (Japan), and Ginn (Canada) in 1986. Molecular identification was performed using ITS sequences. BLAST search in NCBI revealed that the closest match was *P. cerasi* with a 97% identity. The sequences generated represent the first available reference sequences of this species. Timely and accurate identification of the (re)emerging diseases is essential for disease control and protection of Indigenous food-plants.

[P59] Developing cisgenic resistance gene stacks for improved resistance to wheat stem rust disease

Ming Luo¹, Aihua Wang¹, Dhara Bhatt¹, Brenton Brooks¹, Brian Steffenson², Taj Arndell¹, Melania Figueroa¹, Peter Dodds¹, and Mick Ayliffe¹

¹CSIRO A&F, GPO Box 1700, Canberra, Australia; ²Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA

Correspondence to: mick.ayliffe@csiro.au; ming.luo@csiro.au

Fungal rust diseases are a major production constraint in wheat with the combined annual global cost of stem rust, stripe rust and leaf rust estimated to be more than \$3 billion. The most cost effective and environmentally sustainable approach to control rust diseases is via genetic resistance. However, these pathogens evolve rapidly and can quickly overcome resistance genes, particularly when they are deployed individually. Polygenic resistance is believed to provide more durable resistance, however selection of multiple unlinked resistance genes in breeding programs is difficult and expensive and gene combinations quickly separate in future breeding efforts. A more effective strategy for strong, longer-lasting resistance is to combine multiple genes into a single locus with multi-gene cassettes. We have developed wheat lines containing multi-gene

cassettes encoding up to five different stem rust resistance genes at single loci. Furthermore, two separate resistance gene stacks, each encoding five wheat stem rust resistance transgenes, have been combined by conventional breeding to generate wheat plants containing an unprecedented level of poly-transgenic stem rust disease resistance. Next generation stem rust resistance gene stacks that also encode 5 resistance genes have now been produced in wheat using a Precision Engineering approach that incorporates only wheat DNA sequences i.e. no selectable markers or cloning scars. These entirely cisgenic plants have the potential for reduced regulatory burden given recent changes to GM legislation in some countries. This cisgenic technology is applicable to other crops for improving disease resistance and for developing other polygenic traits of agronomic significance with single gene inheritance.

[P60] Flying Towards Healthier Crops: Drone-Based Plant Disease Monitoring

Mehrdad Madani¹, Ying Long², Paul Westdal³, Chris Bunio².

¹Formerly agronomist at ²Ldon Technologies, ³TheoryMesh Ltd, Winnipeg, MB. Canada.

Correspondence to: Madani.Mehrdad@gmail.com

Integrated Pest Management (IPM) strategies and crop health monitoring are essential for food security and sustainable agriculture. Aerial data collection by drones offers noninvasive field scouting and inspection, especially in large farms. Potential use of DJI Mavic 3 Multispectral (M3M) and Matrice 30 Thermal (M30T) drones were evaluated to identify any anomalies due to biotic/abiotic factors in wheat, soybean, and canola fields in southern Manitoba during the 2023 growing season. To assess measurement of thermal gradient and the accuracy, soybean leaves exhibiting varying percentages of necrosis and chlorosis were attached to 60 x180 cm wooden boards and images were captured from altitudes of 5, 10, and 15 m. Ground photos were taken for later comparison and validation (Fig). DJI Thermal Analysis Tool 3 software was used to process over 5000 images in NIR and RGP. This took 24 minutes on average, covering 26437 m². Vegetation indices NDVI, GNDVI and OSAVI, with different thermal palettes, such as IronRed and Tint, revealed distinct boundaries between vegetative greenish parts and areas with anomalies. In a particular wheat field three sections were identified when OSAVI soil index was used for data analysis. Thermal imagery differentiated healthy (22 °C) from necrotic (27.7 °C) leaves. Different reflectance in aerial imagery correlated with biotic or abiotic stress, diseases, pests and nutrient deficiency. Drones provided efficient and rapid data collection, supporting their value in precision agriculture by providing a non-invasive, time- efficient, and resource-saving method. Further research is needed to correlate aerial patterns to specific pathogens through soil and plant testing.

***[P61] Stemphylium leaf spot on Quinoa: A Growing concern in Saskatchewan**

Edgar Mangwende¹, Samira Safari¹, Zoé Lepage¹, Norman Muzhinji², Liam O'Halloran³, Homa Askarian⁴, Alireza Akhavan⁵, Christopher Yost¹

¹Department of Biology, University of Regina, Regina, Saskatchewan, Canada; ²Department of Plant Sciences, Plant Pathology Division, University of the Free State, Bloemfontein, South Africa;

³Northern Quinoa Production Corporation (NorQuin), Saskatoon, Saskatchewan, Canada;

⁴Agriculture Research Branch, Saskatchewan Ministry of Agriculture, Regina, Saskatchewan, Canada; ⁵Crops and Irrigation Branch, Saskatchewan Ministry of Agriculture, Regina, Saskatchewan, Canada.

Corresponding author: Christopher Yost, Email: chris.yost@uregina.ca

Quinoa cultivation in Canada is threatened by the novel emergence of leaf spot diseases. During the 2022 growing season, leaf spot symptoms characterized by black to brown spots were frequently observed on quinoa leaves in Saskatchewan fields. The aim of this study is to identify the responsible pathogen, analyze its morphological and phylogenetic traits, and assess its pathogenic potential and host spectrum across 17 different crops, including beet, canola, faba bean, field beans, lentil, pea, quinoa, red kidney bean, tomato, and wheat. Forty-seven fungal isolates were obtained from affected quinoa foliage displaying pale brown spots. Of these, 24 isolates demonstrated distinct medium to deep brown, oblong conidia with 4–9 septa. Phylogenetic analysis using the ITS, GAPDH, and cmdA gene sequences highlighted a strong relationship with *Stemphylium vesicarium*, with impeccable clustering evidenced by a 100% bootstrap consensus. Infection trials revealed initial light brown lesions primarily at the leaf peripheries of quinoa, escalating to widespread necrosis and decay. While faba beans and lentils emerged as most susceptible, noteworthy vulnerability in quinoa to IMSS269, IMSS273, and IMSS282 isolates was observed. Broad host infection was prominent with isolate IMSS263, which affected 11 of the 14 test plants, showing a high susceptibility in beans and beets, contrary to the resistance in wheat and peas. Isolates IMSS280 and IMSS269 proved highly aggressive on quinoa varieties, unlike IMSS263, which induced subtle infection. The pathogen's successful re-isolation fulfilled Koch's postulates, affirming *S. vesicarium* as the pathogen of quinoa leaf spot. This study elucidates *S. vesicarium*'s pathogenicity and host range in Canada, contributing vital knowledge for effective disease management and future agricultural strategies.

[P62] Effect of temperature and time on survival of *Plasmodiophora brassicae* resting spores in suspension.

Kirsten Holy¹, Bruce D. Gossen², Mary Ruth McDonald¹

¹Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada; ²Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada.

Correspondence to: mrmcdona@uoguelph.ca

The soil-borne Chromist *Plasmodiophora brassicae* (Woronin) causes clubroot disease on brassica crops. The pathogen infects the host to create characteristic 'clubbed' roots, which release resting spores back into the surrounding soil. There have been accounts of resting spore dissemination through water sources, but resting spore survival in water has not been assessed. Resting spores were suspended in sterile water in 50 mL Falcon tubes and kept on the bench top (22°C), refrigerator (4°C) freezer (-20°C), or thawed and refrozen on each sample date, for 0, 2, 4, 8, 16, 32, 75, and 150 days. Resting spore viability was assessed using Evans Blue vital stain. After

each assessment date, resting spores (5×10^5 spores mL⁻¹) were used to inoculate susceptible canola (*B. napus*) line ACS N39 to conduct a bioassay. Evans Blue showed that viability decreased by 15–25% during the first week. Viability decreased gradually over time and was lowest in the freeze/thaw treatment on most assessment days. On day 150, viability was 20% for the freeze/thaw treatment, 27% bench, 45% refrigerator and 49% in the freezer. Clubroot severity in the bioassays was similar among treatments from day 0–32. At day 150, severity was ~30–40% for the freezer and bench treatments and 65% for the refrigerator treatment. The study showed that movement of water from a clubroot-positive field poses a high risk for spread of viable inoculum. These results are also useful for laboratory best-practices; resting spores in water lose some viability within one week.

[P63] Automated treatments with ultraviolet-C radiation control powdery mildew of tomato and cucumber in a fully enclosed environment

Geneviève Marchand, Alyssa Thibodeau, and Quade Digweed

Harrow Research and Development Centre, Agriculture and Agri-Food Canada, 2585 County Road 20, Harrow, ON, Canada, N0R 1G0

Correspondence to: genevieve.marchand2@agr.gc.ca

Automated treatments with ultraviolet-C control powdery mildew of tomato and cucumber in a fully enclosed environment. Renewed interest in food security and the production of fresh food in remote areas and urban centres has led to the rapid expansion of fully enclosed systems for controlled environment agriculture (CEA), in industrialized nations. In Canada, no pesticide products are currently registered for the control of plant pathogens in fully enclosed CEA systems, requiring the use of alternative strategies. Research has demonstrated the efficacy of ultraviolet radiation as a physical method to control foliar plant diseases. Our experimental goal was to explore the potential of a custom, automated system for use in growing containers, to control powdery mildew on fruit and vegetable crops. A custom CEA container system, designed after those already deployed in Gjoa Haven (Nunavut), was designed and built at the Harrow Research and Development Centre and outfitted with commercially available germicidal light fixtures emitting in the ultraviolet-C (UV-C) range. A total of three trials were performed between February 2023 and May 2024. A target dose of 50 $\mu\text{J cm}^{-2}$ was applied two to three times a week to tomato and cucumber crops in hydroponic growing systems, following inoculation with powdery mildew. UV-C treatments had a statistically significant impact on disease severity on both crops. Mild, transient signs of phytotoxicity were occasionally observed on both species. Further research is under way, to fine tune the dosage of UV-C treatments and develop an automated system to control powdery mildew in CEA systems.

[P64] Soilless mix and surfactants affect severity of clubroot (*Plasmodiophora brassicae*)

Komathi Prapagar¹, Merek Wigness², Bruce D. Gossen² and Mary Ruth McDonald¹

¹University of Guelph, Department of Plant Agriculture, 50 Stone Rd E, Guelph, ON N1G 2W1, Canada; ²Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, SK S7N 0X2

Correspondence to: mrmcdona@uoguelph.ca

Clubroot, caused by the soil-borne Chromist *Plasmodiophora brassicae*, is an important disease of canola. Controlled environment studies are often a component of research on this pathogen. Occasionally little or no disease develops in plants grown in some soilless mixes, even when conditions are optimum for infection. Why some mixes do not support clubroot is not known. One hypothesis is that specific surfactants added to these mixes suppress clubroot. Studies were conducted to compare the effect of selected commercial soilless mixes and surfactants on clubroot severity. The soilless mixes were Sunshine LA4 which is conducive for clubroot, compared to BM6 HP and Pro-Mix BX with Mycorrhizae. The surfactants were: nonylphenoxy polyethoxy ethanol 92% (Agral 90), alcohol ethoxylate 19.4% + orange oil 10% (Revonsa Next), saponin extracted from *Yucca schidigera* 20% (ThermX-70 Yucca Extract), propylene glycol 5-7 % (Penterra) and polyalkylene glycol (Duration), all applied at the recommended concentration. The studies were conducted in a growth room using clubroot susceptible canola L233P. Clubroot severity was assessed after 6 weeks. Severe symptoms developed in LA4 (95%) compared to BM6 (12%) and ProMix (3%). Duration did not reduce clubroot severity in any mix. All other surfactants reduced clubroot in LA4, but not to the low levels in nontreated ProMix. Agral 90 reduced severity in LA4 more than the other surfactants. Information on the surfactants in each mix is often proprietary and does not explain all of the differences among the soilless mixes. Clearly, selection of soilless mix is very important for clubroot research.

[P65] Evaluation of the effect of chickpea and flax varietal differences in flax-chickpea intercropping on disease management

Keiko Nabetani¹, Michelle Hubbard², Lana Shaw³, Maria Alejandra Oviedo-Ludena¹, William May⁴, Bunyamin Tar'an¹, Randy Kutcher¹

¹Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N

5A8; ²Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, 1

Airport Road Swift Current, SK S9H 3X2; ³South East Research Farm, Box 129 Redvers, SK S0C 2H0;

⁴Indian Head Research Farm, Agriculture and Agri-Food Canada, #1 Government Road Indian Head, SK S0G 2K0

Chickpea-flax intercropping has been tried and used by several farmers in Saskatchewan and a handful of studies has been conducted for optimization of agronomy practices and evaluation of its benefits. No study has been conducted to evaluate the effects of varietal differences on the success of intercropping, especially with the disease management aspect. The current field study was initiated to evaluate the effect of varietal differences in chickpea-flax intercropping on disease management with *Ascochyta* blight of chickpea and pasmo of flax. Three varieties of chickpea ('CDC Leader', 'CDC Orkney', 'CDC Pearl') were intercropped with each of three flax varieties ('CDC Kernen', 'CDC Glas', 'CDC Sorrel') in RCB with four replicates. The trial was conducted at four sites (Indian Head, Saskatoon, Swift Current, Redvers) in SK in 2023 and 2024. In 2024, Saskatoon and Redvers experienced a substantial *Ascochyta* blight severity. 'CDC Leader' and

'CDC Orkney' had higher Ascochyta blight AUDPC than 'CDC Pearl'. With week 5 of disease assessment, Ascochyta blight severity on 'CDC Orkney' was lower with 'CDC Sorrel' flax than with 'CDC Kernen' flax. At Redvers, 'CDC Leader' had the highest Ascochyta blight AUDPC out of three varieties. All sites except Saskatoon had no pasmo development on flax. At Saskatoon, varieties of flax or chickpea had no effect on pasmo severity. Chickpea yield was affected by both chickpea and flax varieties and was highest with 'CDC Sorrel' flax followed by 'CDC Glas' second and 'CDC Kernen' third at Saskatoon. These trials will continue in the 2025 growing season.

[P66] Timing matters: Revealing phase-specific climate sensitivities in winter wheat using a non-destructive approach

Khadija Sabir¹, Tsu-Wei Chen², and Hartmut Stützel³

¹KWS SAAT SE & Co. KGaA, 37555 Einbeck, Germany; ²Group of Intensive Plant Food Systems, Albrecht Daniel Thaer Institute of Agricultural and Horticultural Science, Humboldt University Berlin, Berlin, Germany; ³Institute of Horticultural Production Systems, Leibniz University Hannover, Hannover, Germany

Correspondence to: khadija.sabir@kws.com

Wheat yield is predicted to decrease globally due to climate change. Unpredictable short-term environmental fluctuations impact the grain yield, depending on the physiological sub-phases of the plant during which they occur. This emphasizes the importance to investigate the three-way interactions between genotype-specific phenology, yield formation and environmental fluctuations to reduce the gap between potential and actual yields under enhanced climatic variability. Experimentally, it is difficult to quantify these effects because such studies are labor-intensive and less-generalizable as the effects of environmental fluctuations are coupled with genotype-specific phenology. Therefore, we developed a novel statistical approach to process data from multi-locational field trials. This approach allowed us to estimate the sensitivities of three yield components (spike number, kernel number per spike, and thousand kernel weight) to variations in global radiation, temperature and precipitation in 220 cultivars across 81 time-windows ranging from double ridge to seed desiccation. We revealed previously undetected environmental sensitivities in specific phases of spike and kernel development, such as the positive effect of night time temperature and global radiation on kernel weight during white anther and canopy senescence, respectively. Further experimental validations confirmed 1) phase-specific sensitivity to environmental fluctuations and 2) genotype-specific strategies to compensate for loss of yield potential due to short-term environmental fluctuation. Our research offers insights into complex genotype-environment interactions, which can be used for genetic and agronomic adaptations to enhance resilience and bridge yield gaps under climate variability.

[P67] Evaluation of disease mitigation effect and optimal fungicide regime with flax-chickpea intercropping system

Keiko Nabetani¹, Michelle Hubbard², Lana Shaw³, Maria Alejandra Oviedo-Ludena¹, William May⁴, Bunyamin Tar'an¹, Randy Kutcher¹

¹Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8; ²Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, 1 Airport Road Swift Current, SK S9H 3X2; ³South East Research Farm, Box 129 Redvers, SK S0C 2H0; ⁴Indian Head Research Farm, Agriculture and Agri-Food Canada, #1 Government Road Indian Head, SK S0G 2K0

Intercropping is a cropping system in which two or more different crops are grown in the same field with some overlap in time. Chickpea-flax intercropping has been one of the common intercropping systems that was utilized by some farmers and studied in Saskatchewan for potential benefits such as accelerated chickpea maturity under wetter conditions, higher land use efficiency and disease mitigation, especially with *Ascochyta* blight of chickpea. The current study was conducted to evaluate efficacy of disease management by chickpea-flax intercropping on *Ascochyta* blight of chickpea and pasmo of flax and the optimal fungicide application regime under the intercropping system. The trials were conducted at four sites (Indian Head, Saskatoon, Swift Current, Redvers) in SK in 2023 and 2024. In 2024, Saskatoon and Redvers experienced a substantial level of *Ascochyta* blight. Indian Head and Swift Current had only trace level of diseases due to a drought condition. At Saskatoon, *Ascochyta* blight severity was lower in intercrop than monocrop while fungicide application had no effect. Although the difference was not significant, *Ascochyta* blight severity tended to be lower in intercrop than monocrop at Indian Head and Redvers. Average pasmo severity was at trace to low levels at all sites. Pasm severity was higher in intercrop than flax monocrop at Indian Head; however, the average severity was very low (<2%). There was no difference in pasmo severity by any treatment at Saskatoon or Redvers. Higher pasmo disease pressure is needed for better evaluation. These trials will continue in the 2025 growing season.

[P68] A critical analysis of adult plant resistance to stripe rust across years and environments in Eastern Africa, Oceania and South Asia

Karanjeet S. Sandhu¹, Davinder Singh¹, Fikrte Y. Belayneh², Tamrat Negash², Hanif Khan³, Subhash C. Bhardwaj³, Suraj Baidya⁴, Dhruva B. Thapa⁴, Muhammad Fayyaz⁵, Shahzad Asad⁵, Sridhar Bhavani and Robert F. Park¹

¹Plant Breeding Institute, University of Sydney, Private Bag 4011 Narellan, NSW 2567, NSW Australia; ²Kulumsa Agricultural Research Centre, Ethiopian Institute of Agricultural Research, Ethiopia; ³Indian Institute of Wheat & Barley Research, Karnal, India; ⁴Nepal Agricultural Research Council, Kathmandu, Nepal; ⁵Crop Diseases Research Institute, Pakistan Agricultural Research Council, Islamabad, Pakistan ⁶International Maize and Wheat Improvement Center (CIMMYT)
Correspondence to: karanjeet.sandhu@sydney.edu.au

Rising demand for wheat requires increased production, but rust diseases especially wheat stripe/yellow rust (WYR) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) threatens productivity. WYR is best managed by resistance, with emphasis on adult plant resistance (APR). Although deemed durable, little effort has been made to examine the performance of APR across different environments. A set of 250 genotypes sourced from 5 countries (Australia, Ethiopia, India, Nepal,

Pakistan) carrying APR to WYR was selected from 3,000 wheat genotypes based on screening for All Stage (ASR) and APR resistance at the Plant Breeding Institute Cobbitty (Australia). Genotyping using molecular markers linked to stripe rust APR genes *Yr18* and *Yr46* revealed that 113 genotypes carried *Yr18* or *Yr46*, and 9 genotypes carried both genes. GWAS of 33 sets of adult plant response data collected from sites in the 5 countries between 2017–2021 and 90K SNP data revealed 20 significant QTL across chromosomes 2A, 3A, 5A, 6A, 7A, 1B, 2B, 4B, 5B and 7B. Although several genotypes were identified that remained consistently resistant across all sites and years, many genotypes that were resistant in Australia were vulnerable to *Pst* at field sites along the Himalayan region in South Asia. The poorer performance of these lines in some environments is concerning. Efforts to understand this are underway, including the development of near isogenic stocks carrying single APR genes and the development of methods to undertake quantitative adult plant phenotyping under controlled conditions.

[P69] Stem rust in Germany, ongoing development and more presence than just on wheat

Philipp Schulz¹, Anne Witzke¹, Nathalie Beilke¹, Tim Kloppe¹, Peng Zhang², Edyta Paczos-Grzęda³
Kerstin Flath¹

¹Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institut für Plant Protection in Field Crops and Grassland, 14532 Kleinmachnow, Germany; ²Plant Breeding Institute, The University of Sydney, Private Bag 4011, Cobbitty, Narellan, NSW 2567, Australia; ³Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

Correspondence to: Philipp.Schulz@julius-kuehn.de

Blumeria f.sp. and *Puccinia graminis* are continuous threats for agriculture and therefore related diseases are part of the German varietal evaluation within the registration process. As relative “new”, more precise re-emerging disease stem rust need to be considered in Germany as additional problem, for which precaution need to be taken. Since 2013, the first outbreak of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) the disease appear nearly annually and we will show that several genetic clades are established and that there is continuous evolution, with the recent appearance and characterisation of an isolate virulent to Sr31. With this, there is no by German isolates unbroken resistance gene in the German varieties anymore. Next to stem rust in wheat, we show that rye stem rust (*Puccinia graminis* f. sp. *secalis*) is a considerable problem and evolving dynamic as shown by genetic and differential tests, both demonstrating a high degree of complexity. Furthermore, Pgs harbours the potential to spill over in other crops, particular barley as we have recently shown. As the latest re-addition to the stem rust family in Germany oat stem rust (*Puccinia graminis* f. sp. *avenae*) was identified. This the first time since 30 years and collaborative effort demonstrated an increase in virulence compare to the previous described isolates.

[P70] QTL mapping of stripe rust resistance in a winter wheat recombinant inbred line population

Mitra Serajazari¹, Ana Laura Achilli², Mehrdad Abbasi², Gurcharn S. Brar³, Curt McCartney⁴ and Helen Booker¹

¹University of Guelph, 50 Stone Road East, Guelph, ON, Canada, N1G 2W1; ²University of British Columbia, Vancouver, BC, Canada, V6T 1Z4l ³University of Alberta, 116 St & 85 Ave, Edmonton, AB, Canada, T6G 2R3; ⁴University of Manitoba, 66 Chancellors Cir, Winnipeg, MB, Canada, R3T 2N2
Correspondence to: mserajaz@uoguelph.ca

Stripe rust, caused by *Puccinia striiformis* (*Pst*), has expanded into new regions of North America. In 2016, Ontario experienced an unprecedented stripe rust epidemic in winter wheat, likely due to more aggressive and heat-tolerant pathogen races. This study evaluated a recombinant inbred line (RIL) population derived from a cross between 'Priesley' (resistant) and 'Venture' (susceptible) winter wheat cultivars from Ontario, Canada. The population was assessed for seedling and adult-plant resistance against *Pst* isolates UGWYr16001 and W053 from the University of Guelph and the University of British Columbia, respectively. Disease infection type (0–9 scale) was recorded at the seedling stage, and infection response (0–9 scale) was measured at the adult-plant stage. DNA from 242 individuals, including parents, was analyzed using Genotyping-by-Sequencing (GBS) at Laval University's Genomic Analysis Platform. A linkage map was constructed using MapDisto 2.1.8, incorporating 9,631 SNPs. Linkage groups were identified using a logarithm of the odds (LOD) threshold of 3.0, and distorted markers were excluded. Two genic regions were identified on chromosomes 2A and 7D, associated with seedling and adult-plant resistance, respectively. These regions encompass known resistance genes *Yr1* and *Yr18*. Future research will focus on fine-mapping these regions to identify candidate genes and develop molecular markers for breeding rust-resistant wheat.

[P71] A Colombian wheat breeding line possesses adult plant resistance to stem rust on chromosome 1B, which is distinct from Sr58

Davinder Singh^{1*}, Urmil Bansal¹, Peace Kankwatsa², Karanjeet S. Sandhu¹, Kerrie Forrest³, Robert F. Park¹

¹Plant Breeding Institute, School of Life and Environmental Sciences, University of Sydney, Private Bag 4011 Narellan, NSW 2567, Australia; ²National Agricultural Research Organization, Mbarara-ZARDI, Uganda; ³Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083.
*Correspondence to: davinder.singh@sydney.edu.au

Puccinia graminis f. sp. *tritici* (*Pgt*), a devastating rust pathogen causing stem rust (SR) in wheat, poses a major threat to productivity by diminishing yield and quality. Resistance breeding is the most effective management strategy to mitigate SR, and researchers seek new sources of resistance continuously to diversify breeding programmes. In previous studies, we identified a Colombian breeding line (accessioned as AUS12578) with good levels of field resistance to SR. Multi-pathotype seedling greenhouse tests using 10 diverse Australian *Pgt* pathotypes revealed the presence of the seedling resistance gene *Sr12* in AUS12578. Despite using *Sr12*-virulent

pathotypes, AUS12568 showed good levels of resistance (20-30MRMS) in the field suggesting the presence of additional adult plant resistance (APR). To characterise and map this APR, we developed a RIL mapping population (n=99) derived from AUS12578 and CDAV245 (a line highly susceptible to stem rust). The population was phenotyped over two seasons and genotyped using a targeted genotype by sequencing (tGBS) assay for 11k exome SNPs. QTL mapping identified a major QTL (tentatively designated *QSr_Aus12578*) on chromosome 1BS, significantly associated with APR and explaining 14.7% of phenotypic variance. Markers associated with *QSr_Aus12578* were located at 1.2–3.6Mb (in Chinese Spring reference genome v1.1), a genomic region distinct from *Sr58/Lr46/Yr29* locus on chromosome 1B. Two closely linked KASP markers were developed for this locus, with sunKASP_530 being highly polymorphic and suitable for marker-assisted selection. The marker was used to genotype 95 Australian wheat cultivars, 7 of which (Bonnie Rock, Cobra, Dart, Giles, Janz, Livingstone and Wedgetail) tested positive.

[P72] A Decade of wheat leaf rust surveillance reveals seven new races of *Puccinia triticina* in South Africa

Tarekegn Terefe¹, Botma Visser², Zacharias Pretorius², and Willem Boshoff²

¹Agricultural Research Council - Small Grain, Private Bag X29, Bethlehem 9700, South Africa; ²Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa

Correspondence to: terefet@arc.agric.za

Wheat cultivars resistant to *Puccinia triticina* (*Pt*) provide an ecofriendly approach to leaf rust control in South Africa (SA). Leaf rust monitoring helps to timeously detect new *Pt* races, assess their epidemic potential and impact on cultivar responses. Diversity of *Pt* was analyzed in SA from 2013 to 2022 using standard differentials and microsatellite (SSR) markers. Fourteen *Pt* races were identified from 1122 isolates pathotyped, with CFPSJ, CDPSK, CFPSK, MCPSK, MFPSJ, MFPSK, and CNPSK being detected for the first time. Most of the new races exhibited combined virulence to multiple resistance genes compared to the old races. Increased virulence frequencies were noted on resistance genes *Lr1*, *Lr20*, *Lr24* and *Lr26*. Further, CNPSK defeated the previously effective *Lr9* resistance gene in SA. Six of the seven new races were virulent on more than 55% of the 131 wheat entries evaluated, CFPSK being the most virulent (69%). Certain cultivars showed increased susceptibility to new races, e.g., SST 0166 to MFPSJ and MFPSK, Tredou to MCPSK, MFPSJ and MFPSK, and SST 0117 to CNPSK. Results of SSR and virulence analyses indicated that the new races CFPSJ, CFPSK, MCPSK and MFPSJ may have developed locally through mutation, whereas CDPSK and MFPSK were believed to be exotic introductions.

[P73] Isolation and Characterization of Endophytic *Schizophyllum commune* AM804 Strain and its Biocontrol Potential Against Phytopathogenic Fungi

Xinmei Xu¹, Siyuan Zheng¹, Richard Hamelin², and Yongjun Wang¹

¹Department of Forest Protection, College of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, 666 Wusu Street, Lin 'an, Hangzhou, Zhejiang Province,

China, 311300; ²Department of Forest and Conservation Sciences, Faculty of Forestry, The University of British Columbia, 2424 Main Mall, Vancouver, BC, Canada, V6T 1Z4

Correspondence to: xuxinmei32@gmail.com

Fungal pathogens are major threats to plants and fruits, leading to substantial agricultural losses worldwide. Endophytic fungi, particularly those producing volatile organic compounds (VOCs), represent a promising source of natural biological control agents (BCAs). This study aimed to isolate a novel Volatile-producing endophytic fungus (VPE) with the most effective biocontrol activity and to investigate its antifungal mechanisms. A total of 131 endophytic fungi were isolated from different tissues of common subtropical aromatic trees, including *Prunus mume*, *Osmanthus fragrans*, and *Cinnamomum camphora*. These isolates were classified into 27 genera, comprising 90.07% *Ascomycota* and 9.92% *Basidiomycota*. VOCs from each isolate were screened for antifungal activity against four pathogenic fungi using a dual-culture plate method. One isolate, the basidiomycete *Schizophyllum commune* (AM804), exhibited strong antifungal activity, *inhibiting Sclerotinia sclerotiorum*, *Botryosphaeria dothidea*, *Phytophthora cinnamomi*, and *Penicillium digitatum* by 97.37%, 56.05%, 41.59%, and 73.37%, respectively. In vivo fumigation with AM804 VOCs reduced *P. digitatum* infection in citrus fruits by 80.0%. Headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME- GC/MS) identified the major compounds as methyl 2-methylbutyrate (43.16%), 3-methyl-1-butanol (8.74%), and ethyl 2-methylbutanoate (6.49%). Both in vitro EC₅₀ assays and in vivo fumigation experiments confirmed the antifungal efficacy of these compounds. Among possible mechanisms of action, AM804 was found to inhibit spore formation and germination, suppress hyphal elongation, and disrupt cell membrane integrity. Overall, AM804 represents a promising BCA for managing both plant fungal diseases and postharvest fungal infections in citrus fruits.

[P74] Virulence and Genetic Diversity in *Bipolaris sorokiniana* population from Western Canada

[Xiben Wang](#)¹, Kelly Turkington², Rehmadi Sajid³, Randy Kutcher⁴, Stephen Strelkov⁵ and Zhen Yao¹

¹Agriculture and Agri-Food Canada, Morden Research and Development Centre, Morden, MB, R6M1Y5, Canada; ²Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, MB, T4L 1W1, Canada; ³Western Crop Innovations, Lacombe, MB, T4L 1W1, Canada; ⁴Crop Development Centre/Department of Plant Science, University of Saskatchewan, Saskatoon, SK, S7N5A8, Canada; ⁵Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada

Correspondence to: xiben.wang@agr.gc.ca

Spot blotch, caused by *Bipolaris sorokiniana*, is one of the most important leaf spot diseases of barley in the Canadian prairies. To effectively deploy genetic resistance against this pathogen, it is crucial to understand the genetic variability of *B. sorokiniana* and the interactions between *B. sorokiniana* and barley. This study investigated the virulence diversity of *B. sorokiniana* isolates collected during the annual barley disease survey in the three western Canadian provinces in 2023 and 2024. The virulence of these *B. sorokiniana* isolates was analyzed using a set of differentials

containing 20 barley genotypes. In addition, we sequenced 32 *B. sorokiniana* isolates exhibiting high pathogenic variation for a comparative genomic analysis to understand the factors governing their aggressiveness in causing spot blotch of barley. The result of the virulence and genetic diversity analysis will aid in design and improvement of barley-breeding programs to develop new cultivars and facilitate the development of effective and durable management strategies to control barley spot blotch.

[P75] iTAG training: Interactive laboratory exercises to explore genotype and phenotype using Oregon Wolfe barley

Roger P Wise^{1,2}, Gregory Fuerst¹, Nick Peters², Nancy Boury², and Laurie McGhee³

¹U.S. Department of Agriculture-Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011; ²Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, IA 50011 ³Colfax-Mingo Community High School, 204 N League Rd, Colfax, IA 50054

Correspondence to: Roger.Wise@USDA.gov

iTAG (Inheritance of Traits and Genes) uses the morphogenetically diverse Oregon Wolfe Barleys (OWB) in laboratory and classroom activities to connect visible traits (phenotype) to identifiable differences in the DNA sequence. iTAG focuses on 3-5 traits to illustrate basic concepts in plant development, domestication, and disease resistance, with the ultimate objective to train researchers to understand and monitor a few genes as a foundation for tracking and leveraging hundreds to thousands as they engage with modern genomics later in their careers. Using the iTAG barley module, students observe the OWB spikes for seed shape and color, two row vs. six row (*Vrs1*), hooded vs. non-hooded (*Kap*), and long awn vs. short awn (*Lks2*). Lastly, the OWB population segregates for resistance or susceptibility to powdery mildew disease, due to different alleles of *Mildew locus a (Mla)*. Participants learn basic molecular biology techniques of DNA extraction, polymerase chain reaction, gel electrophoresis, and document DNA polymorphisms among plants with different phenotypes. Instructors can then lead a discussion of how researchers associate genotype and phenotype. Thus, new researchers gain valuable experience in genetic history related to cellular pathways and developmental mutations; concepts critical to producing disease resistant crops and livestock, as well as human health. iTAG barley has been used successfully by >50 instructors across the USA in >200 high school and community college biology classes from 2010 to 2024, impacting nearly 5000 students, of which one third were from underrepresented groups from urban to rural communities. iTAG curriculum is freely available at: <https://www.apsnet.org/edcenter/learningPP/Pages/PHI-E-2023-09-0009.aspx>

[P76] Genetic structure of North American *Puccinia coronata* f. sp. *avenae* (the cause of crown rust in oats) populations

Bharat Yadav¹, Samuel Holden¹, Jujhar Gill¹, Naveen Kumar¹, James G. Menzies³, Kirby T. Nilsen², and Gurcharn S. Brar¹

¹Faculty of Land and Food System, The University of British Columbia – Vancouver, British Columbia, Canada; ²Agriculture and Agri-Food Canada – Brandon, Manitoba, Canada; ³Agriculture and Agri-Food Canada – Morden, Manitoba, Canada
Corresponding author: Gurcharn S. Brar (Gurcharn.brar@ualberta.ca), Kirby T. Nilsen (kirby.nilsen@agr.gc.ca)

Oat crown rust, caused by the fungus *Puccinia coronata var avenae* f.sp. *avenae* (*Pca*), is a major production problem for oat farmers in Canada – with consequences ranging from yield loss upto 40 percent. Understanding the genetic diversity of the pathogen population is essential for deciphering its adaptation and evolution in diverse hosts and environments. This study aimed to identify the genetic diversity of *Pca* in Canada through high-throughput sequencing. The primary objective was to assess the genetic distinctiveness of reproducing clonal individuals in the eastern prairie region (Manitoba and eastern Saskatchewan) compared to sexually reproducing individuals in eastern Canada (Ontario and Quebec). We used 141 isolates in this analysis, including 81 *Pca* isolates sampled in Canada, sequenced through Illumina NovaSeq6000, and short NGS data from 60 US isolates. To address genetic differentiation among the *Pca* isolates four key tools were employed, incorporating parametric (PCA) and non-parametric (DAPC) clustering approaches, as well as model (RAxML-NG) and non-model-based (STRUCTURE) structural analyses. Population genetic analysis uncovered a relatively low level of genetic variation ($F_{ST} = 0.0093$) in the Canadian *Pca* population, indicative of limited diversity within Canadian isolates. We found distinct genetic patterns emerged based on geographical regions, notably the eastern prairie region and eastern Canada, with discernible patterns of genetic differentiation over time. A temporal comparison between the US population and the Canadian *Pca* populations revealed notable differences. Overall, the US population showed moderate ($F_{ST} = 0.068$) internal genetic variation but still differed from the Canadian *Pca* population. This study enhances our understanding of the genomic diversity of *Pca* in Canada and the US, providing critical insights into its evolution and adaptation. These findings lay the groundwork for developing effective strategies to combat oat crown rust in North America.

[P77] Protein-protein network hubs in host-pathogen interactions: Targets for next-generation breeding

Roger P. Wise^{1,2}, Valeria Velásquez-Zapata², Shivansh Patel³, Schuyler Smith^{3,4}, John Higgins^{3,4}, Zoi Goebel³, Gregory Fuerst¹, and J. Mitch Elmore⁵

¹USDA-Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011; ²GreenLight Biosciences, Inc. Research Triangle Park, NC 27709;

³Department of Plant Pathology, Entomology and Microbiology, Iowa State University, Ames, IA 50011; ⁴Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831; ⁵USDA-Agricultural Research Service, Cereal Disease Laboratory, 1551 Lindig Street, St. Paul, MN 55108

Correspondence to: Roger.Wise@USDA.gov

Disease phenotypes are the result of dynamic changes in gene and protein interactions at multiple levels in multiple cellular compartments. To establish a regulatory network view of protein-protein

interactions (PPI) critical to pathogen infection and disease resistance in cereals, we constructed PPI networks of barley (*Hordeum vulgare* L.) in response to powdery mildew, caused by the ascomycete fungus, *Blumeria hordei* (*Bh*). The barley MLA nucleotide binding, leucine-rich repeat (NLR) receptor was used as a model regulator to interrogate host immune response, as its alleles and orthologs confer recognition specificity to diverse fungal diseases, including powdery mildew, stem rust, stripe rust, spot blotch, and rice blast. On the pathogen side, 48 representative *Bh* effector proteins, including AVR_{A1}, AVR_{A6}, AVR_{A7}, AVR_{A9}, and AVR_{A13}, were selected from time-course RNA-sequencing on barley CI 16151 progenitor and fast-neutron immune mutant derivatives. Next, all MLA domains and *Bh* effectors were used as baits in triplicated yeast two-hybrid next-generation interaction screens, where batch matings with a 3-frame infection prey library were followed by quantitation and ranking of individual Illumina 20-million read samples via project-developed NGPINT and Y2H-SCORES software, and subsequent binary confirmation. Results were integrated with the HvInt barley interactome, enabling assembly of a high-confidence host-pathogen network of 1085 proteins and 1497 interactions to further probe cellular localization and immune activation for next-generation breeding to new and emerging pathogens.

[P78] Molecular diagnostics and fungicide efficacy for control of powdery scab in Alberta

Muhammad Subhan Shafique¹, Michele Konschuh¹, Jennifer Foster³, Michael Harding² and Dmytro Yevtushenko¹

¹Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4;

²Alberta Agriculture and Irrigation, Brooks, AB, Canada, T1R 1E5; ³Syngenta Canada Inc., Guelph, ON, Canada, N1G 4Z3

Correspondence to: dmytro.yevtushenko@uleth.ca

Powdery scab is a soil-borne disease of potato, caused by the obligate fungus-like protozoan *Spongospora subterranea* f. sp. *subterranea*. This pathogen causes root galls and tuber skin blemishes, and serves as the sole known vector for potato mop-top virus (PMTV) that causes spraing in the tuber flesh. In this study, seven field sites with a known history of powdery scab were selected; the presence of the pathogen was confirmed by PCR amplification of the ITS region (ITS1-5.8S-ITS2) in DNA samples isolated from the soil of these sites. The study was conducted over three consecutive growth seasons (2022–2024), using potato cultivars Shepody, Russet Burbank and Lady Claire, planted in naturally infested soils. Five Syngenta-designed fungicide treatments, namely A21008A, Allegro (low, medium and high) and A24367B, were evaluated for their effectiveness to protect plants from powdery scab. In 2023, Allegro was the most effective in reducing root galls, followed by A24367B, whereas A21008A performed poorly. However, A21008A showed the highest efficacy in reducing tuber lesions. All treatments significantly suppressed the disease in Lady Claire. In the 2024 trials, none of the treatments suppressed galls in Russet Burbank, whereas all but Allegro-low significantly reduced galls in Lady Claire. No significant differences in total tuber yield were observed across treatments or cultivars. In bioassays, conducted by growing plants in the presence of controlled quantities of *S. subterranea*, visible symptoms of powdery scab were observed when soil contained more than 15 cystosori per gram, which may indicate a potential threshold for disease development.

[P79] Stripe rust fungi hijack host sugar transporters for enhanced nutrition acquisition in wheat

Peijing Zheng¹, Pu Yuan², Nannan Fang¹, Xiaoxuan Ma², Lihua Jiang², Zhensheng Kang², and Jie Liu^{1*}

¹State Key Laboratory for Crop Stress Resistance and High-Efficiency Production and College of Life Sciences, Northwest A&F University, Yangling 712100, Shaanxi, China

²State Key Laboratory for Crop Stress Resistance and High-Efficiency Production and College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China

Correspondence to: liujie2003@hotmail.com

Biotrophic pathogens rely on host plants for carbon acquisition, making plant sugar transporters, such as Sugars Will Eventually be Exported Transporters (SWEETs), key targets in the sugar competition between plants and pathogens. In this study, we identified TaSWEET14d, tightly associated with stripe rust resistance by GWAS analysis, as a SWEET transporter. TaSWEET14d was significantly induced during *Puccinia striiformis* f. sp. *tritici* (*Pst*) infection of wheat. Transient expression and mutant complementation indicated that TaSWEET14d was localized to secreted vesicles and has broad substrate specificity. Interestingly, TaSWEET14d-targeted secretory vesicles were found to tightly surround fungal haustoria, and some GFP signals appeared to be localized to the extrahaustorial membrane. Knockout of *TaSWEET14d* using CRISPR/Cas9-based gene editing led to increased wheat resistance to *Pst*, while overexpressing *TaSWEET14d* promoted stripe rust susceptibility and sugar accumulation in wheat leaves. Additionally, transcription factor TaMYB50 was shown to contribute to stripe rust resistance by suppressing TaSWEET14d expression via binding directly to the TA-box motif in the promoter region. Furthermore, we found that effector protein Pst15882, required for *Pst* infection and pathogenicity, interacted with TaMYB50 to prevent this repression. Taken together, our findings reveal that *Pst* hijacks SWEET transporter TaSWEET14d exploiting effector protein Pst15882 to interfere with TaMYB50-mediated transcriptional suppression, effectively enhancing fungal access to host sugars for successful invasion, and suggested TaSWEET14d as a potential target to improve stripe rust resistance.

[P80] The highly effective crown rust resistance gene *Pc101* in oat has been mapped to chromosome 2D

Edyta Paczos-Grzeda, Sylwia Okoń, Sylwia Sowa

Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka Str. 15, 20-950 Lublin, Poland

Correspondence to: edyta.paczos@up.lublin.pl

Crown rust caused by *Puccinia coronata* Corda f. sp. *avenae* (P. Syd. & Syd.) (*Pca*), is one of the most destructive fungal diseases of oats worldwide and represents a major constraint to oat production. Breeding disease-resistant oat cultivars is the most effective strategy for controlling the spread of crown rust and preventing potential epidemics. Currently, over 100 crown rust

resistance (Pc) genes have been identified in oat, however, the chromosomal locations of most of these genes remain unknown. The objective of this study was to genetically map the position of the *Pc101*, a race-specific seedling crown rust resistance gene that confers effective resistance at all growth stages. *Pc101* was derived from *Avena sterilis* accession PI 334961, originally collected in Haifa, Israel. Physiological tests of crown rust response were conducted, and segregation ratios in the population derived from a cross between *Pc101* differential line and the crown rust susceptible Polish cultivar ‘Kasztan’ confirmed monogenic inheritance of the resistance gene. Linkage analysis to identify a map location for *Pc101* was performed in bi-parental mapping population genotyped with DArTseq™ markers generated by Illumina next-generation short-read sequencing. Markers linked to *Pc101* were mapped to chromosome 2D of the *Avena sativa* cv. Sang reference genome, within the region spanning 170 Gb and 174 Gb. Based on SNPs identified in the DArTseq sequences derived from this chromosomal interval, allele-specific markers were developed. These newly developed markers represent valuable tools for marker-assisted selection in oat breeding programmes and may facilitate the pyramiding of multiple resistance genes.

[P81] Genetic Differentiation and Virulence Spectrum of *Blumeria graminis* f. sp. *avenae*

Tomasz Ociepa^{1,2}, Sylwia Okoń¹, and Aleksandra Nucia¹

¹Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15, 20- 950 Lublin, Poland; ²Department of Bioinformatics, Polish-Japanese Academy of Information Technology, Koszykowa 86, 02-008 Warsaw, Poland

Correspondence to: tomasz.ociepa@up.lublin.pl, sylwia.okon@up.lublin.pl

Understanding the genetic diversity and virulence profiles of plant pathogens is crucial for studying host-pathogen interactions. In this study, 166 single spore *Blumeria graminis* f. sp. *avenae* (*Bga*) isolates, collected from five European countries, were analyzed. High-density SNP data were generated through DArTseq genotyping and processed using the R package dartR, with subsequent genetic distance analyses including PCoA, heatmap, and dendrograms. Marker data underwent quality filtering based on reproducibility (>98%), call rate (>95%), and MAF (>1%). Virulence of analysed *Bga* isolates was independently assessed via host-pathogen tests on a panel of 14 oat differential lines. The scoring was done on a scale of 0 to 4, where 0 indicates no infection and no visible symptoms, while 4 represents high susceptibility with abundant sporulation. Genetic structure analysis revealed significant differentiation among *Bga* isolates, primarily correlated with their geographic origin. A distinct genetic cluster was identified, primarily comprising isolates from Poland. Analysis of phenotypic virulence profiles on differential lines with various Pm resistance genes demonstrates variability in virulence among isolates and populations. Virulence complexity (number of overcome resistance genes) and frequency of virulence per line were calculated from these phenotypic data. Combining genetic and phenotypic findings revealed potential links between genetic backgrounds and virulence profiles, with certain genetically distinct isolates and populations showing unique virulence characteristics.

[P82] Genetic characterization of powdery mildew resistance in oat landraces: phenotypic screening and GWAS insights

Sylwia Okoń¹, Kathy Esvelt Klos², Belayneh Yimer², Aleksandra Nucia¹, Tomasz Ociepa¹

¹Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15, 20- 950 Lublin, Poland; ²USDA-ARS, Small Grains and Potato Germplasm Research Unit, 1691 S 2700 W, Aberdeen, ID83210

Correspondence to: sylwia.okon@up.lublin.pl, kathy.klos@usda.gov

Powdery mildew, caused by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *avenae* (Bga), represents a significant threat to oat (*Avena sativa* L.) production. Annual outbreaks of the disease occur with varying intensity across many regions worldwide, contributing to yield losses estimated between 10% to 40%. In recent years, environmental fluctuations have contributed to the spread of the disease to new geographic areas. Moreover, the adaptive nature of the continuously evolving pathogen population has led to the breakdown of resistance conferred by previously described *Pm* resistance genes. Although thirteen *Pm* genes have been identified in oats, only a few are currently utilized in breeding programs providing effective resistance. Genetic resistance remains the primary strategy for powdery mildew control in oats, hence new sources of resistance are continually sought to counter the adaptation of the pathogen populations. Therefore, this study aimed to evaluate 671 genotypes, comprising landraces and historical cultivars, from the National Small Grains Collection (NSGC) for resistance to powdery mildew. Resistance evaluation was conducted at the seedling stage using host-pathogen interaction tests with three *Bga* isolates differing in virulence and geographical origins. Genome-wide association mapping (GWAS) was performed using the oat 6K Illumina iSelect SNP-chip to identify QTL linked associated with powdery mildew resistance. A subset of genotypes with high resistance to powdery mildew was identified, and GWAS analysis revealed QTLs linked to powdery mildew resistance. These findings highlight the potential of NSGC landraces and historical cultivars as valuable sources of novel powdery mildew resistance genes.

[P83] The role of ncRNAs as potential molecular regulators of oat resistance to *Blumeria graminis* and *Puccinia* spp. Infections

Tomasz Ociepa^{1,2}, Sylwia Okoń¹, Michał Nowak¹ and Wiktoria Jędrys¹

¹Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15, 20- 950 Lublin, Poland; ²Department of Bioinformatics, Polish-Japanese Academy of Information Technology, Koszykowa 86, 02-008 Warsaw, Poland

Correspondence to: tomasz.ociepa@up.lublin.pl

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play key roles in post-transcriptional and epigenetic regulation of gene expression in plants. They are involved in developmental processes, hormone perception, and response to environmental stresses, including pathogen attacks. While specific information on ncRNAs in oat resistance to *Blumeria graminis* (powdery mildew) and *Puccinia* spp. (rusts) is limited, literature reports on other crops suggest their potential importance. Studies on wheat have revealed differential expression of lncRNAs in response to *Blumeria graminis* f. sp. *tritici* infection, with some functioning as miRNA precursors. Similarly, research has identified lncRNAs responsive to *Puccinia striiformis* f. sp. *tritici*

infection, suggesting regulatory roles in plant defense. Despite the lack of oat-specific data, findings from wheat and other plants indicate that ncRNAs regulate plant immune responses to fungal pathogens. Considering the evolutionary conservation of miRNAs, similar mechanisms likely function in oats, influencing resistance to powdery mildew and rusts. This presentation summarizes current findings on ncRNAs in cereal resistance to fungal pathogens and outlines our research plan to identify miRNA and lncRNA sequences in transcriptomes of oat infected by *Blumeria* and *Puccinia* pathogens, contributing to understanding molecular mechanisms of resistance in this plant.

[P84] Inhibitory effects of coumarin derivatives on biotrophic fungal pathogens in cereals

Klaudia Rząd¹, Aleksandra Nucia², Arkadiusz Matwijczuk¹, [Sylwia Okoń](#)²

¹Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland; ²Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland

Correspondence to: sylwia.okon@up.lublin.pl

Obligatory biotrophic fungi such as *Blumeria graminis* (powdery mildew) and *Pucciniales* (rusts) threaten global cereal production. These pathogens occur annually, causing significant yield losses. In addition, they can quickly adapt and develop resistance to previously used pesticides, reducing their effectiveness. Challenges in cereal cultivation resulting from the presence of fungal pathogens have forced the agricultural sector to search for new, effective and safe plant protection products. One of the groups studied in this respect is natural coumarin and its derivatives. This group shows, among others antifungal properties and good biodegradability. This makes them a promising candidate for the development of new fungicides. Presented study aimed to determine the potential of coumarin derivatives in inhibiting the growth and development of the most important biotrophic cereal pathogens. The biological activity of the compounds studied was analyzed *in vitro* using physiological host-pathogen tests. Leaves of susceptible cereal varieties (oats, wheat, triticale, barley) were placed on Petri dishes containing agar medium supplemented with coumarin derivatives. Leaf fragments were inoculated with spores of fungal pathogens, and after 10 days of incubation, the degree of infection of leaf fragments was determined. The study results showed that coumarin derivatives had varying effects on the growth of biotrophic fungal pathogens. However, most of them were effective in inhibiting the growth of fungal pathogens. The research allowed the identification of a group of derivatives with a broad spectrum of biological activity, which can be used in further research on the development of new, effective and ecological agrochemicals.

[P85] Further assessment of new seed treatments against blackleg in canola

Xiaohan Zhu¹, Chun Zhai¹, Wali Soomro¹, Yong Min Kim and [Gary Peng](#)¹

¹Saskatoon Research and Development Center, ²Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, SK S7N 0X; ²Brandon Research and Development Center, AAFC, 2701 Grand Valley Road, Brandon, MB R7A 5Y3

Correspondence to: gary.peng@agr.gc.ca

Early fungicide applications can significantly reduce blackleg impact on canola when cultivars are highly susceptible; however, their cost-effectiveness is limited due to minimal yield benefits in resistant cultivars. Succinate dehydrogenase inhibitors (SDHIs), such as fluopyram and pydiflumetofen, have shown promise as seed treatments at labeled rates of 75 g and 40 g a.i./100 kg seed, respectively. However, results from large-scale plot and strip trials have been inconsistent. This study evaluated the efficacy of fluopyram at different rates in protecting canola cotyledons and lower true leaves from blackleg. Acibenzolar-S-methyl (BION® 500FS), a plant activator, and pydiflumetofen (Saltro®) were included for comparison at rates of 18.75 mL and 40 g a.i./100 kg seed, respectively. Greenhouse trials demonstrated that fluopyram at 75 g a.i./100 kg seed significantly reduced infection development on inoculated cotyledons and its spread to the stem, thereby lowering blackleg incidence and severity ($P < 0.002$, ANOVA). However, field trials in 2021 and 2022 failed to confirm this efficacy against blackleg originating from cotyledon or lower true-leaf inoculation ($P > 0.05$, GLM), whereas the BION treatment was significantly effective against cotyledon inoculation only ($P < 0.05$). In contrast, field trials in 2023 and 2024 demonstrated that fluopyram at both 75 g and 150 g a.i./100 kg seed reduced blackleg incidence and severity originating from cotyledon inoculation ($P < 0.05$). Saltro also showed comparable efficacy in these trials. These findings suggest that SDHI and BION seed treatments may help manage early blackleg infections, though their effectiveness is limited to the cotyledon stage only and may vary by year or environment.

*[P86] Exploring wheat stripe rust resistance – can wheat relatives play a role?

Jujhar Singh Gill¹, Vincent Fetterley¹, Gurcharn Singh Brar^{1,2}

¹Faculty of Land and Food System, The University of British Columbia - Vancouver, British Columbia, Canada

²Faculty of Agricultural, Life, and Environmental Sciences, University of Alberta, Edmonton, Canada

Correspondence to: gurcharn.brar@ualberta.ca

Stripe rust of wheat, caused by *Puccinia striiformis*, is a devastating fungal disease that disrupts photosynthesis and nutrient absorption, reducing crop yield and quality. In severe outbreaks, yield losses can reach 70%, threatening global food security. Genetic resistance is the most sustainable and preferred control strategy, but the rapid evolution of *P. striiformis* and defeat of previously deployed resistance genes necessitates the continuous discovery of novel resistance sources. While resistance has been extensively studied in bread wheat (*Triticum aestivum*), research on its wild relatives remains limited. This study investigates spelt wheat (*Triticum spelta*), a wild relative of bread wheat, as a potential source of novel resistance genes. We employ Bulk-Segregant Analysis (BSA) to identify resistance loci in a spelt wheat mapping population by comparing allelic frequencies between resistant and susceptible bulks. Plants are inoculated with *P. striiformis* and phenotyped in climate-controlled growth chambers, followed by DNA extraction and genotyping. By correlating the generated genotypic and phenotypic data, we aim to identify bulks with extremely contrasting phenotypes and expect to identify single nucleotide polymorphisms (SNPs) that segregate with resistance, allowing us to approximate the genomic region conferring resistance. These findings will provide a foundation for further fine-mapping of the resistance loci, gene cloning and marker-assisted breeding, facilitating the development of stripe rust-resistant

wheat varieties. Ultimately, this work supports global food security and enhances agro-economic resilience through improved disease management strategies.

***[P87] Uncovering Stripe Rust Resistance Loci and Markers via Nested Association Mapping in Spring Wheat**

Simranjeet Kaur^{1,2}, Raman Dhariwal², Gurcharn S. Brar¹, and Harpinder S. Randhawa²

¹University of Alberta, 116 St & 85 Ave, Edmonton, AB, Canada, AB T6G 2R3

²Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403 1 Ave S, Lethbridge, AB, Canada, T1J 4B1

Correspondence to: simranj9@ualberta.ca

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), poses an evolving and devastating threat to global wheat production. The emergence of virulent races belonging to the *PstS1* lineage has rendered previously resistant cultivars susceptible, underscoring the need for durable, broad-spectrum resistance. In this study, we employed a nested association mapping (NAM) strategy to dissect the genetic basis of stripe rust resistance in spring wheat. Three NAM populations were developed by crossing the susceptible cultivars Avocet, Vesper, and Cardale with 16 genetically diverse and highly resistant donor lines, resulting in 16 recombinant inbred line (RIL) families. Individual QTL mapping of each RIL population, along with joint QTL mapping, identified several novel and known QTL distributed across multiple chromosomes. These loci exhibited consistent effects across environments, advancing our understanding of the genetic architecture of stripe rust resistance. The integration of individual RIL population data and joint QTL mapping enabled the identification of key resistance loci with broader applicability across diverse breeding contexts. These findings also facilitated the identification of tightly linked molecular markers, which are crucial for marker-assisted selection in wheat breeding programs.