

growing ideas

November 20 - 22

PROGRAM



8th Canadian Workshop
on Fusarium Head Blight

8^e Colloque canadien
sur la fusariose



Ottawa
Ontario, Canada

8th Canadian Workshop on Fusarium Head Blight

8^e Colloque canadien sur la fusariose

Delta Ottawa City Centre

Ottawa, Ontario, Canada

November 20-22, 2016

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Welcome

It is with pleasure that I welcome you all to Ottawa for the 8th Canadian Workshop on Fusarium Head Blight (CWFHB)!

With fusarium head blight rapidly spreading to all three Prairie provinces in western Canada in the last five years, managing this disease remains a top priority for Canada. And a similar problem exists in many temperate regions of the world.

The 8th CWFHB provides a forum for stakeholders, including researchers, producers, grain handlers, end users, consumers and regulators, to discuss the latest Canadian and international progress on addressing the problems caused by *Fusarium* species in cereal crops. Highlights of the recent developments in breeding for resistance to FHB, in genomics and genetics of host and pathogen, in epidemiology and disease management, and with mycotoxin issues are presented.

It is hoped that the oral and poster presentations, as well as the social events, will provide networking opportunities that will foster the development of collaborative action plans to address the economic and safety issues associated with fusarium head blight.

I would like to close by expressing my gratitude to the organizers from SeCan, to the program organizing committee and to the sponsors for their generous contributions.

Thérèse Ouellet
Chair, 8th CWFHB
Ottawa Research and Development Centre
Agriculture and Agri-Food Canada

Bienvenue

C'est avec plaisir que je vous souhaite la bienvenue à Ottawa pour le 8^{ème} Colloque Canadien sur la Fusariose (CCF) !

Avec la fusariose qui s'est propagée rapidement aux trois provinces des prairies canadiennes dans les cinq dernières années, la gestion de cette maladie demeure une priorité principale pour le Canada. Et un problème analogue existe dans plusieurs régions tempérées du monde.

Le 8^{ème} CCF offre une tribune aux intervenants, incluant les chercheurs, producteurs, manutentionnaires de grains, utilisateurs finaux, consommateurs et régulateurs, pour discuter des derniers développements au Canada et internationalement vers la résolution des problèmes causés par les espèces de *Fusarium* dans les grandes cultures céréalières. L'accent est sur les faits nouveaux en amélioration de la résistance à la fusariose, en génomique et génétique de l'hôte et du pathogène, en épidémiologie et gestion de la fusariose, et avec les problèmes associés aux mycotoxines.

J'ai espoir que les présentations orales et en affiches, ainsi que les activités sociales, apporteront des opportunités de réseautage qui vont favoriser le développement de plans d'action coopératifs visant à pallier les conséquences économiques et sur la sécurité associées avec la fusariose.

Je voudrais finir en exprimant ma reconnaissance envers les organisateurs de chez SeCan, le comité d'organisation du programme et les commanditaires pour leurs contributions généreuses.

Thérèse Ouellet
Présidente, 8^{ème} CCF
Centre de recherche et développement d'Ottawa
Agriculture et Agroalimentaire Canada

Welcome from SeCan

On behalf of SeCan's 700 member companies across Canada, I would like to welcome you all to the 8th Canadian Workshop on Fusarium Head Blight. I would also like to say what an honour it has been to assist our colleagues at AAFC to organize this very important event.

As a national association of small independent seed companies, SeCan represents the largest supplier of certified seed to Canadian farmers - and of that – wheat is the single most important crop. Wheat is one of the few crops that is grown in every province of Canada, and unfortunately, is now suffering from increasingly severe fusarium infestations in all major production areas. As recently as this 2016 harvest, our western Canadian based member seed companies are forecasting fusarium to be the major determining factor in wheat production decisions for 2017. And in some cases, it will knock wheat completely out of their crop rotation plans. The devastating infection of 2016 once again demonstrates the dramatic impact fusarium has on our farm gate income – not to mention food safety concerns for the balance of the food processing value chain.

So it is against this backdrop of the need for urgent progress against this disease that we hope you will engage in a very fruitful exchange of knowledge, and build collaborations that will move us closer to a solution for this most challenging disease. Again, thank you on behalf of SeCan companies across Canada for the work you do – and for your participation in this important workshop.

Bienvenue de SeCan

Au nom des 700 entreprises membres de SeCan au Canada, j'aimerais vous souhaiter la bienvenue au 8^{ème} Colloque Canadien sur la Fusariose. Je tiens également à dire à quel point ce fut un honneur d'avoir aidé nos collègues d'AAFC à organiser cet événement des plus importants.

En tant qu'association nationale de petites entreprises semencières indépendantes, SeCan représente le plus grand fournisseur de semences certifiées auprès des agriculteurs canadiens, dont le blé est la culture la plus importante. En effet, le blé est l'une des quelques cultures à être cultivées dans chaque province du Canada; malheureusement, cette culture est affligée par des problèmes de plus en plus sévères de fusariose dans toutes les grandes zones de production. Avec la récente récolte de 2016, nos entreprises membres de l'Ouest canadien prévoient que la fusariose constituera le plus grand facteur déterminant dans les décisions prises en matière de production de blé en 2017. Et, dans certains cas, cette maladie éliminera complètement le blé de leurs plans de rotation culturale. L'infection dévastatrice de 2016 démontre encore une fois l'impact dramatique que la fusariose a sur le revenu à la ferme, sans mentionner les préoccupations relatives à la salubrité alimentaire qu'elle suscite pour le reste de la chaîne de valeur de transformation des aliments.

C'est donc dans ce contexte marqué par l'urgence de faire des progrès contre cette maladie que nous espérons que vous vous engagerez dans un fructueux échange de connaissances et établirez des collaborations qui nous rapprocheront d'une solution face à ce fléau. Encore une fois, je vous remercie au nom des entreprises membres de SeCan partout au pays pour le travail que vous accomplissez et pour votre participation à cet important colloque.

Jeff Reid
General Manager | Directeur général



Exhibitors

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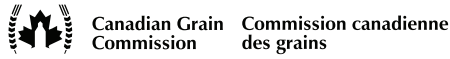
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8th Canadian Workshop on Fusarium Head Blight

Sunday 20 November 2016

18:00 – 21:00	Opening Reception Sponsor: Sask Wheat Development Commission	Pinnacle Room
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Monday 21 November 2016

7:30 – 8:30	Breakfast	Ballroom A&B
8:30 – 8:50	Welcome	Ballroom A&B
Plenary Lectures		Ballroom A&B
8:50 – 9:25	<i>Overview on FHB1</i> Jim Anderson	
9:25 – 10:00	<i>Biology and Management of Fusarium graminearum: Lessons Learned from Close Encounters with the Toxic Foe</i> Gary Bergstrom	
10:00 – 10:30	Morning Break	Ballroom C
Cereal Genomics & Genetics Co-chairs: Michele Loewen and Nora Foroud		Ballroom A&B
10:30 – 11:00	<i>Genomic Approaches to Developing FHB Resistance in Wheat and Barley</i> Gary Muehlbauer	
11:00 – 11:15	<i>Genomics and Genetics for FHB Resistance in Bread Wheat, Durum Wheat and Triticale</i> Thomas Miedaner	
11:15 – 11:30	<i>Barley Chitin Elicitor Receptor Kinase (HvCERK1) Confers Resistance Against Fusarium graminearum</i> Dhananjay Dhokane	
11:30 – 11:45	<i>Characterization of Novel FHB Resistance QTL in Canadian Wheat Using a Joint Multiple Population Approach</i> Pierre Fobert	
11:45 – 12:00	<i>Collective Effort to Develop Genomics and Genetics Tools for the Cloning of the FHB-Resistance Locus from Thinopyrum elongatum Chromosome 7EL</i> Thérèse Ouellet	
12:00 – 13:00	Lunch	Ballroom A&B
Pathogen Genomics & Genetics and Population Biology Co-chairs: Gopal Subramaniam and Tom Graefenhan		Ballroom A&B
13:00 – 13:30	<i>Elucidating the Secrets of Life Cycle of Fusarium graminearum</i> Frances Trail	
13:30 – 13:47	<i>Comparative Population Genomics of Fusarium graminearum Reveals Adaptive Divergence among Cereal Head Blight Pathogens</i> Todd Ward	
13:47– 14:04	<i>Whole Genome Sequencing and Comparative Genomics of Fusarium Head Blight Fungi</i> Sean Walkowiak	
14:04 – 14:21	<i>Examining Fusarium Species Interactions in Durum Wheat</i> Linda J. Harris	
14:21 – 14:38	<i>Characterization of a Secreted Fusarium graminearum Elongation Factor – An Elicitor of Host Defence?</i> Nora A. Foroud	

14:38 – 15:05	Afternoon Break <i>Water Station Sponsor: Richardson</i>	Ballroom C
Update on Mycotoxins Co-chairs: Barbara Blackwell and Sheryl Tittlemier		Ballroom A&B
15:05 – 15:35	<i>What's DON got to do with it? Measurement and Management of Mycotoxins in Malting Barley from Field to Glass</i> Aaron MacLeod	
15:35 – 15:55	<i>Maximum Levels for DON in Cereals and Cereal Product: Updates on CODEX and Health Canada Activities</i> Elizabeth Elliott	
15:55 – 16:15	<i>Targeted Surveys of Deoxynivalenol in Domestically Produced and Imported Foods Available on Canadian Store Shelves</i> Beata Kolakowski	
16:15 – 16:35	<i>Richardson International – Supplying Food to the World</i> Lynne Sweeney	
16:35 – 18:00	Poster Session <i>Sponsor: KWS LOCHOW GMBH</i>	Ballroom C

Tuesday 22 November 2016

7:30 – 8:30	Breakfast <i>Sponsor: Canadian National Millers Association</i>	Ballroom A&B
Resistance Breeding A Co-chairs: Richard Cuthbert and Ron Knox		Ballroom A&B
8:30 – 9:00	<i>FHB in Brazil – Challenges, Successes and Future Work</i> Igor Pirez Valério	
9:00 – 9:30	<i>Breeding for Wheat Germplasm with Enhanced FHB Resistance and Reduced DON Content at CIMMYT</i> Xinyao He	
9:30 – 9:45	<i>FHB Resistance Breeding in Durum Wheat: Progress and Perspectives</i> Yuefeng Ruan	
9:45 – 10:00	<i>Breeding for Enhanced Fusarium Head Blight Resistance in Canadian Bread Wheat</i> Santosh Kumar	
10:00 – 10:30	Morning Break	Ballroom C
Resistance Breeding B Co-chairs: Richard Cuthbert and Ron Knox		Ballroom A&B
10:30 – 10:45	<i>Breeding Winter Wheat for Fusarium Head Blight Resistance – Challenges and Progress</i> Ljiljana (Lily) Tamburic-Ilincic	
10:45 – 11:00	<i>Improving FHB Resistance in Canadian Barley: Recent Progress and Future Prospects</i> Bill Legge	
11:00 – 11:15	<i>Fusarium Head Blight of Oat – Progress in Dealing With a Sly Foe</i> Jennifer Mitchell Fetch	
11:15 – 11:30	<i>proWeizen – The German Wheat Research and Breeding Alliance</i> Tanya Gerjets	
11:30 – 11:45	<i>Identification of Genomic Regions Associated with Resistance to Fusarium Head Blight, Leaf Rust and Stem Rust in Winter Wheat</i> Silvia Barcellos Rosa	
11:45 – 11:55	<i>FHB can be Defeated by a Systemic Approach</i> André Comeau	
11:55 – 12:00	General Discussion/Questions	
12:00 – 13:00	Lunch	Ballroom A&B

Epidemiology & Disease Management		Ballroom A&B
Co-chairs: Maria Antonia Henriquez and Allen Xue		
13:00 – 13:40	<i>Managing Fusarium Head Blight: Successes and Future Challenges</i> Ruth Dill-Macky	
13:40 – 14:00	<i>Cropping Factors: The Key for Sustainable Mycotoxin Management in Cereals</i> Susanne Vogelgsang	
14:00 – 14:20	<i>DONguard: Suppression to Control Through Microfloral Shift and IPM</i> Bill Brown	
14:20 – 14:40	<i>Metabolomics Analysis of the Effect of Elevated CO₂ on Wheat Resistance to Fusarium Head Blight</i> Miroslava Cuperlovic-Culf	
14:40 – 15:00	<i>Prevalence of Fusarium spp. Causing Head Blight of Spring Wheat, Barley and Oat in Ontario During 2001 – 2015</i> Allen Xue	
15:00 – 15:30	Afternoon Break <i>Water Station Sponsor: Western Grains Research Foundation</i>	Ballroom C
Meeting Food Safety Requirements and Consumer Expectations		Ballroom A&B
Chair: Gordon Harrison		
15:30 – 17:00	<i>Discussion Panel</i> Gordon Harrison Susan Abel	
17:30 – 18:00	Bus Transfer to Banquet <i>Sponsor: FP Genetics</i>	Ballroom Lobby
18:00 – 19:00	Cocktail Reception <i>Sponsor: Bayer</i>	Museum of Nature
19:00 – 21:30	Banquet <i>Dinner Sponsor: SeCan</i> <i>Wine Sponsor: Bayer</i> <i>Entertainment Sponsor: Crop Development Centre</i>	

ABSTRACTS FOR ORAL PRESENTATIONS

Plenary lectures

S-01

Overview on Fhb1

Nidhi Rawat¹ Bikram S. Gill² and James A. Anderson³

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²Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

³Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

Fhb1 is a major quantitative trait locus (QTL) providing partial resistance to fusarium head blight (FHB) of wheat. Mapped to the short arm of chromosome 3B nearly 20 years ago, the phenotypic effects of Fhb1 include limiting fungal spread following infection by *Fusarium* species and detoxification of the fungal toxin deoxynivalenol (DON). Reduction of FHB symptoms by 20-25% are typically attributed to Fhb1. Experiments aimed at map-based cloning of Fhb1 were first reported in 2003 and fine mapping activities resulted in the development of diagnostic markers for use in selection. Comparison of DNA sequences of BAC clones from Fhb1-containing germplasm and the Chinese Spring reference genome indicate that the Fhb1 region contains several unique genes. Efforts to clone Fhb1 have been hampered by a lack of i) recombination in a 350 kb region containing 13 Fhb1 candidate genes; and ii) clear differential expression pattern among candidates. Following validation by induced mutation, gene silencing, and transgenic over-expression, the gene responsible for the reduced fungal spread of Fhb1 has been identified as a pore-forming toxin (PFT). The PFT gene is predicted to encode a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain and this function is consistent with its durable, broad-spectrum nature. However, unlike lines lacking Fhb1, PFT mutant lines were able to detoxify DON when directly applied to spikes. Therefore, we believe that Fhb1 is a complex of two or more closely linked genes and a gene responsible for DON detoxification has yet to be identified.

S-02

Biology and management of *Fusarium graminearum*: Lessons learned from close encounters with the toxic foe

Gary C. Bergstrom

Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853-5904 USA

The speaker relates lessons learned and questions still in need of answers from his years of research and extension experience with *Fusarium graminearum* in the contexts of cereal production in the Northeastern US, service with the US Wheat and Barley Research Initiative, and international collaboration on the epidemiology of the pathogen. Research at Cornell pioneered new understandings of the aerobiology of the pathogen and the prospects for management with cultural practices in predominately maize-producing regions. New York is an excellent living laboratory for study of the population biology of the pathogen; fungal populations exhibit both a latitudinal and a longitudinal cline through New York in the proportion of trichothecene chemotypes. Current research seeks to better understand the factors driving natural selection in populations of *F. graminearum* in agricultural fields as well as natural grasslands that interface with cereal production in the state. Integrated management remains the only viable approach. Moderate resistance is the bedrock of management programs but such varieties are still deployed on less than half of vulnerable cereal acres. Growers' perceptions and understanding of risk are key to changing this situation. Resistance to triazole fungicides in fungal populations is a sleeping giant that must be managed proactively before the effectiveness of this essential class of fungicides is lost to cereal producers. Biological control could become a more useful management tool following investment in formulation and application technologies. Finally the speaker relates challenges ahead in producing low DON malting barley in humid, nontraditional areas such as New York.

Cereal Genomics & Genetics

S-03

Genomics approaches to developing FHB resistant wheat and barley

Gary J. Muehlbauer

Department of Plant Biology, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

Fusarium head blight (FHB) is a disease of cereal crops that causes severe yield losses and trichothecene mycotoxin contamination of grain. The main causal pathogen, *Fusarium graminearum*, produces either deoxynivalenol (DON) or nivalenol (NIV) as virulence factors. Type II resistance is conferred by the ability to detoxify trichothecenes and therefore identifying genes that encode enzymes that detoxify DON and NIV may result in tools that can be used to develop resistant genotypes. Barley exhibits an inherent high level of type II resistance. Thus, we sought to identify genes in barley that confer resistance to trichothecenes. Using a combination of genomics and biochemical approaches, we identified a barley UDP-glucosyltransferase, *HvUGT13248* that confers resistance in yeast and *Arabidopsis* to DON-containing media. Expression of *HvUGT13248* in transgenic wheat resulted in high levels of type II resistance in the greenhouse and high levels of resistance in field trials. Several lines exhibited resistance in the greenhouse and field trials that was equivalent to Sumai3. Noteworthy, we expressed *HvUGT13248* in two wheat genotypes and both exhibited high levels of resistance. We also observed that transgenic barley overexpressing *HvUGT13248* exhibited resistance to DON inhibited root growth. *HvUGT13248* confers resistance by rapidly metabolizing DON with a glucoside group to form the less toxic DON-3-O-glucoside. We also observed that *HvUGT13248* confers type II resistance in transgenic wheat to NIV-producing *Fusarium* strains and rapidly metabolizes NIV to the less toxic NIV-3-O-glucoside. These results demonstrate that *HvUGT13248* exhibits high levels of resistance to at least two trichothecenes and that *HvUGT13248* is a useful tool for developing resistant genotypes.

S-04

Genomics and genetics for FHB resistance in bread wheat, durum wheat and triticale

Thomas Miedaner, Hans Peter Maurer and Friedrich Longin

State Plant Breeding Institute, University of Hohenheim, Fruwirthstr. 21, 70599 Stuttgart, Germany

Fusarium head blight (FHB) in bread wheat, durum wheat, and triticale is a serious concern in Europe because of the possible mycotoxin contamination of food and feed. Critical values of deoxynivalenol, zearalenone, T2- and HT2-toxins in food are highly regulated by the European Union. We analyzed elite breeding populations of triticale (N=650), durum wheat (N=184), and bread wheat (N=455 and 1,749) for FHB resistance, plant height, and heading date in multi-environmental trials with artificial infection. In all populations, dwarfing alleles (*Ddw1b* in triticale, *Rht-B1b* in durum, *Rht-B1b+D1b* in bread wheat) increased FHB susceptibility substantially. Because all durum and about 70% of bread wheat cultivars in Central Europe possess one dwarfing allele, populations for selecting both FHB resistance and shortness simultaneously must be large. In all cereals, only small-effect QTL were found in our mapping populations. In European bread wheat >150 QTL are reported locating on nearly all chromosomes that can be merged into 19 metaQTL. Genomic prediction (GP) in durum did not provide much higher predictive ability than marker-assisted selection (0.70 vs. 0.65) while in bread wheat accuracy substantially increased when using GP (0.2 vs. 0.6) in unrelated test and training sets. Genetic relationship further increases accuracy. Phenotypic selection always had the highest accuracy being, however, also most expensive and slower. In conclusion, substantial progress in FHB resistance can be made by both, pure phenotypic selection or genomic selection. The main challenge is not breeding FHB-resistant cultivars, but breeding cultivars that are accepted by the market.

S-05

Barley chitin elicitor receptor kinase (*HvCERK1*) confers resistance against *Fusarium graminearum*

Shailesh Karre¹, Dhananjay Dhokane¹, Arun Kumar^{1,2}, and Ajjamada Kushalappa^{1*}

1 Department of Plant Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada, H9X 3V9

2 Current address: Department of Horticulture, University of Wisconsin - Madison, Madison, USA, 53706

Fusarium head blight (FHB) is a devastating and alarming disease that affects barley and other small cereal grains. FHB not only cause severe losses in yield but also reduces quality of the grains by contaminating them with mycotoxins, thus making them unsuitable for human and animal health. Breeding for resistance is considered to be the best option to manage FHB. Resistance in barley to FHB is quantitative in nature, involving cumulative effects of many genes governing resistance. The incomplete understanding of genetics and lack of precise phenotyping has hindered the development of FHB resistant cultivars. Hence, an attempt was made to identify candidate gene(s) and to elucidate the resistance mechanisms induced by barley resistant genotype CI9831 based on integrated metabolo-transcriptomics. Metabolic profiling of CI9831 upon *Fusarium graminearum* and water (control) inoculations detected high fold changes in metabolites belonging to phenylpropanoid, hydroxycinnamic acid (HCAA) and jasmonic acid pathways. Transcriptome analysis detected high up-regulation of chitin elicitor receptor kinase (*HvCERK1*), MAP kinase 3 (*HvMPK3*), MAPK substrate 1 (*HvMKS1*), *HvERF1/5*, *HvNAC42*, *HvWRKY23* and *HvWRKY70*. Polymorphism studies across three barley genotypes confirmed the presence of mutations in *HvCERK1* gene in two susceptible genotypes, considering *HvCERK1* as a potential candidate conferring FHB resistance. Further, silencing of functional *HvCERK1* gene in the resistant genotype CI9831, followed by gene expression studies and metabolite analysis revealed not only its role as an elicitor recognition receptor but also as a plausible signal transduction gene that triggered downstream regulatory genes, which, in turn, regulated downstream genes to biosynthesize resistance related (RR) metabolites to contain the pathogen to initial infection.

S-06

Characterization of novel FHB resistance QTL in Canadian wheat using a joint multiple population approach

Kerry Boyle¹, Wentao Zhang¹, Anita Brûlé-Babel², George Fedak³, Peng Gao¹, Zeinab Robleh Djama^{3,4}, Brittany Polley¹, Richard Cuthbert⁵, Harpinder Randhawa⁶, Robert Graf⁶, Pierre R. Fobert^{1,4}

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6 Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, T1J 4B1

Fusarium head blight (FHB) is a devastating fungal disease of small-grain cereals that results in severe yield and quality losses. The most effective solution to address FHB is to develop resistant cultivars. However, the quantitative genetic basis of FHB resistance coupled with strong environmental effects has provided serious challenges in achieving this goal. FHB resistance can be divided into a number of components such as incidence, severity, index, DON accumulation and FDK that may be combined into elite cultivars to obtain desired levels of resistance. Recently, three populations were created by crossing FL62R1, an Eastern Canadian spring wheat line with novel FHB resistance, to two CWRS wheat varieties, Stettler and Muchmore, and a winter wheat variety, Emerson, which also possesses effective, novel FHB resistance. These populations were phenotyped for multiple FHB resistant component traits at two locations for two years. They were also assessed for type II resistance (spreading) under greenhouse conditions. Although none of the spring-type parents possessed strong type II resistance, a large proportion of the progenies displayed type II resistance comparable to the resistance standard Sumai3. Progenies with FHB resistance comparable to Sumai3 in disease nurseries were identified. With a high density genetic map generated using 90K SNP arrays and a joint-multiparental QTL mapping approach, QTL for FHB resistance were identified, with 'native QTL' from elite Canadian cultivars and novel QTL from FL62R1 and Emerson identified for different components of FHB resistance. The G x E interactions on FHB resistance will also be discussed.

S-07

Collective effort to develop genomics and genetics tools for the cloning of the FHB-resistance locus from *Thinopyrum elongatum* chromosome 7EL

Thérèse Ouellet¹, Lulu Gou², David Konkin³, Jiro Hattori¹, Farideh Tekieh¹, Danielle Wolfe¹, Margaret Balcerzak¹, Firoozeh Chalabian¹, Aparna Haldar¹, Jan Vrana⁴, Marie Kubalaková⁴, Jaroslav Dolezel⁴, Nicholas A. Tinker¹, Andrew Sharpe³, George Fedak¹.

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² Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China.

³ Aquatic and Crop Resource Development, National Research Council Canada, Saskatoon, SK, S7N 0W9.

⁴ Institute of Experimental Botany, Slechtitelu 31, Olomouc, CZ-78371, Czech Republic

The wild grass species *Thinopyrum elongatum* has been identified as a source of strong fusarium head blight (FHB) resistance. Using wheat addition lines carrying single *Th. elongatum* chromosomes, resistance to FHB has been mapped to the long arm of chromosome 7E. This resistance is of great interest because it protects wheat against FHB to a high level, and also because the source is located on a single chromosome arm, possibly at a single locus, in contrast to other sources of resistance that are complex and multi-genic. A collaborative effort led to the development of approaches and tools to characterize and clone the 7EL source of FHB resistance, including: 1) identification of expressed 7EL candidate genes from contrasting expression profiles between the wheat lines Chinese Spring (CS; FHB susceptible) and CS-7EL (CS plus the addition of the long arm of chromosome 7E); 2) assembly of a draft genome sequence of the 7EL chromosome fragment, obtained using paired end and mate pair Illumina sequencing libraries; 3) development of genetic markers for the expressed 7EL candidate genes as well as pairs of genetic markers from homoeologous regions on 7EL and 7DL genomic DNA; 4) characterization of introgressed wheat families containing fragments of 7EL of different sizes, inserted by recombination into the wheat chromosome 7D, following a cross between the mutant line CSph1b and the substitution line CS-7E(7D).

Pathogen Genomics & Genetics and Population Biology

S-08

Elucidating the secrets of the life cycle of *Fusarium graminearum*

Frances Trail

Michigan State University, East Lansing, MI

The focus of our research is to understand the interaction of *Fusarium graminearum* with its environment across the entire year. Each stage of the life cycle has implications in survival, sporulation, and dispersal, and thus affects disease outcomes. The formation of perithecia and launching of ascospores provides the initial inoculum, along with sporodochia and rain-splash dispersal of conidia. Recent studies have shown details of the infection process through trichomes on floral surfaces. The fungus moves through the flowers into the rachis mainly through the vascular system, and in wet conditions will sporulate through stomates and silica cells. Colonized culms host the fungus over the winter, and provide substrate for sporulation in the spring. With our increasing understanding of the role of microbial communities in plant health, interactions with microbial communities at all of these stages could have implications for disease progression. Continuing to increase our knowledge of the life cycle and interactions with the environment, and use of this knowledge to inform development of control strategies, provide our best chance for disease reduction.

S-09**Comparative population genomics of *Fusarium graminearum* reveals adaptive divergence among cereal head blight pathogens**

Todd J. Ward and Amy Kelly

United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois, USA

During the last decade, a combination of molecular surveillance and population genetic analyses have significantly altered our understanding of *Fusarium graminearum*, the major FHB pathogen in North America. In addition to the native NA1 population (largely 15-ADON toxin type) and the invasive NA2 population (largely 3-ADON toxin type), which has rapidly increased in frequency in some areas, isolates with a novel trichothecene toxin type (NX-2) were recently found to cause FHB in the northern U.S. and southern Canada. In this study, we sequenced the genomes of 60 *F. graminearum* isolates to understand how NX-2 isolates relate to the previously characterized NA1 and NA2 populations; and to identify potential adaptations that distinguish the various populations of *F. graminearum* responsible for FHB in the U.S. and Canada. Genome-wide patterns of SNP diversity revealed that most isolates with the NX-2 toxin type represent a novel genetic population (termed NA3), although genetic exchange among populations was documented. The three genetic populations were found to differ in gene content, with 122 genes showing population-specific patterns of gene conservation. An additional 16 loci, varying in size from 10-40 kb exhibited patterns of adaptive divergence between pathogen populations. Functional annotation of these population-differentiating genes and genomic regions indicated that *F. graminearum* populations in North America harbor unique sets of adaptations that contribute to differences in how these pathogens exploit the agricultural landscape.

S-10

Whole genome sequencing and comparative genomics of fusarium head blight fungi

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The most common fungal species that causes fusarium head blight (FHB) of wheat in Canada is *Fusarium graminearum*. These fungi reduce yields and contaminate crops with the toxin deoxynivalenol (DON). DON has different derivatives, including the acetylated forms 3-ADON and 15-ADON, which have slightly different chemical structures. The population structure of fungi that cause FHB is dynamic; reports indicate that fungi that produce 3-ADON are becoming more abundant in Canada compared to fungi that produce 15-ADON. The question remains: why are some FHB pathogens more successful than others? We believe that the answer to this question lies within differences in the genomes of these fungi. Through whole genome sequencing and comparative genomics, we identified regions of the genome that are changing/different, as well as genes that are absent in certain genomes. Some of the absent genes include a set of 20 genes in one region of the genome of a 3-ADON producing isolate, which are less common in 15-ADON producing isolates. We performed a broader investigation of these genes including their origin, distribution across the genus and species, predictions of gene functions, and expression in wheat.

S-11

Examining *Fusarium graminearum* and *Fusarium avenaceum* interactions in durum wheat

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Fusarium graminearum and *F. avenaceum* are known to co-contaminate Canadian durum wheat samples, leading to FHB and grain contaminated with multiple mycotoxins. Each of these species produces its own diverse set of mycotoxins and other secondary metabolites, of which only a small proportion are overlapping. For example, while *F. graminearum* produces trichothecenes, zearalenone, and culmorin, *F. avenaceum* produces enniatins, moniliformin, and antibiotic Y. To study what happens when these two species encounter each other in the host plant, *F. graminearum* and *F. avenaceum* strains were inoculated individually and in combination in durum wheat spikes and monitored over 7 days. We assessed visual disease symptoms, estimated species-specific fungal biomass, and determined DON concentrations. Overall, the interaction of these two species led to reduced disease and DON levels compared to *F. graminearum* inoculations alone. Global transcriptome analysis of *F. graminearum* exposed to *F. avenaceum* secondary metabolites in liquid culture revealed that a *F. graminearum* ABC transporter gene is highly induced when *F. graminearum* is exposed to purified enniatin B1. This transporter gene is only induced *in planta* when *F. graminearum* is co-inoculated with *F. avenaceum*. The *F. graminearum* ABC transporter protein has been successfully expressed in yeast (*Saccharomyces cerevisiae*) for functional studies and has been shown to rescue yeast from enniatin toxicity. We are investigating what role this transporter may play in interspecies interactions.

S-12

Characterization of a secreted *Fusarium graminearum* elongation factor—an elicitor of host defence?

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Elongation factor 1-alpha (EF1a) was previously identified in the secretome of *Fusarium graminearum* cultured in trichothecene-inducing medium. Under these conditions, secreted proteins are predicted to reflect those that are secreted during the infection process in host plants, and are candidate elicitors of host-defence responses. Eukaryotic EF1a is highly conserved in the plant kingdom and is responsible for coordinating aminoacyl-tRNA positioning in the ribosome during peptide bond formation. Its prokaryotic counterpart, EF-thermo unstable (EF-Tu), is also a well characterized pathogen-associated molecular pattern (PAMP). PAMPs are elicitors of plant defence known to interact with host-cell pattern recognition receptors (PRRs), which detect foreign signatures characteristic of pathogens. Sequence analysis of EF-Tu from a handful of bacterial species showed 30-33% sequence identity with EF1a from a number of plant species. While the prokaryotic EF-Tu is sufficiently foreign to eukaryotic species to elicit plant defense responses, it is not known if the same is true for fungal eukaryotic EF1a which share roughly 70-80% identity with their homologues in plants. Here, a *F. graminearum* mutant overexpressing FgEF1a was found to have significantly reduced aggressiveness by both point and spray inoculation in five wheat cultivars. In highly susceptible 'Roblin' disease was reduced by 60.7% and 77.4% in point and spray inoculated plants, respectively, compared to the wild-type. In highly resistant cultivars 'CM82036' and 'Tenacious', reduction in visual symptoms were also observed in the point inoculated spikelets. Molecular characterization of FgEF1a and analysis of the effect of this protein on plant host defense will be presented.

Update on Mycotoxins

S-13

What's DON got to do with it? Measurement and management of mycotoxins in malting barley from field to glass.

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Significant quantities of malting barley are affected by fusarium head blight each year, limiting the amount available for malting and brewing use. Presence of the *Fusarium* fungus is known to have detrimental effects on the quality of malt and the brewing process, and has been implicated in phenomena such as gushing and premature yeast flocculation. The primary method of screening malting barley for selection purposes is by DON measurement using rapid screening kits, due to the relative ease and low cost. However, recent studies have shown that measurement may be complicated by antibody cross reactions and presence of masked mycotoxins. Other research into the fate of mycotoxins during the malting and brewing process has revealed that mycotoxin contamination can increase during the malting process, which involves raising the moisture content of the grains and increasing the humidity of the environment. As mycotoxins are chemically stable, even during storage and processing when exposed to high temperatures such as those reached during baking bread or breakfast cereal production, they tend to persist to the final product. Surveys have shown that a variety of mycotoxins have been found in commercially available beers at trace levels. Since there are few proven methods for decontaminating the grain, the best method of control found to date is prevention.

S-14

Maximum levels for DON in cereals and cereal products: updates on Codex and Health Canada activities

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The Food Directorate of Health Canada is primarily responsible for the regulation of mycotoxins in foods sold in Canada. It establishes policies and standards, provides information on food safety and administers the provisions of the *Food and Drugs Act* and the *Food and Drug Regulations (FDR)*. Health Canada actively participates in international committees focusing on consumer health protection, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Codex Committee on Contaminants in Food (CCCF); the JECFA conducts human health risk assessments that support the CCCF's work to develop international food safety standards and codes of practice. In 2015, the Codex Alimentarius Commission adopted maximum levels (MLs) for deoxynivalenol in: 1) raw cereal grains (wheat, maize and barley) destined for further processing (2 mg/kg); 2) flour, meal, semolina and flakes derived from wheat, maize or barley (1 mg/kg); and 3) cereal-based foods for infants (< 12 months) and young children (< 36 months) (0.2 mg/kg, dry matter basis). Codex MLs are generally considered for their applicability and potential adoption in Canada, on a case-by-case basis. Health Canada is in the process of reviewing and updating the Canadian MLs for DON, including the existing MLs for DON in soft wheat that were established in the 1980's. Any new MLs for DON in foods that are to be sold in Canada will be included in the *List of Contaminants and Other Adulterating Substances in Foods*, which is incorporated by reference into section B.15.001 of Division 15 of the *FDR*.

S-15

Targeted surveys of deoxynivalenol in domestically produced and imported foods available on Canadian store shelves

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The Canadian Food Inspection Agency (CFIA) is dedicated to safeguarding food, animal, and plant health, which enhances the health and well-being of Canada's people, environment, and economy. The CFIA delivers inspection services to prevent and manage food safety risks and contribute to market access for Canadian agricultural products. Targeted surveys complement the Agency's regular monitoring programs.

Deoxynivalenol (DON) is a non-carcinogenic, pre-harvest fusarium mycotoxin with chronic and acute health effects. Since 2009, 11,144 samples of a wide variety of domestically produced and imported foods were collected from grocery stores across Canada and analyzed for DON. Of these, DON was detected in 61% of samples (6810) at levels ranging from 0.01 to 5720 ng/g. The concentration and frequency of detection differed depending on product category.

The product categories included: milled grain products (e.g. wheat bran); processed grain products (e.g. cookies); pulses/pulse products; and assorted foods (e.g. dried fruits, soy products, infant formula, wine). DON was detected in 81% of processed food, 53% of milled grain products, 12% of pulses/pulse products, and 10% of samples from the assorted food category. The highest observed concentrations were: milled grain products (5720 ng/g); processed grain products (2060 ng/g); assorted foods (26.7 ng/g); and pulses/pulse products (15.5 ng/g).

There are no regulatory limits for DON in most of the products examined. Follow-up actions were taken by the CFIA based on the magnitude of associated health risk as evaluated by Health Canada's Bureau of Chemical Safety. No product recalls occurred as a consequence of these test results.

S-16

Richardson International - Supplying Food to the World

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Whether providing access to leading technologies through modern highly efficient farm business centers, partnering in the development of preventative agronomic plans to aid in the defense of crop disease and pest pressures, investigating crops as early identification of agronomic challenges, collecting representative grain samples from newly harvested fields for analysis of quality, functionality and food safety attributes, or managing for on farm stored grain risk, Richardson is a proactive partner of the Canadian producer in their pursuit of growing a quality, food-safe crop.

As a handler, processor and supplier of grains, oilseeds, ingredients and consumer food products, Richardson's comprehensive risk-based Quality & Food Safety systems serve to mitigate risk and maintain compliance with regulatory standards and commercial commitments, when delivering quality, food-safe products to domestic and international markets.

In addition to holding HACCP GMP B3 and GFSI (FSSC 22000) certification and maintaining compliance with international Food Safety Management System standards, Richardson's integrated risk management strategy carries throughout its grain handling, food processing and export terminal facility businesses, with emphasis on hazard analysis and preventative controls for the management of physical, chemical, biological, adulteration and traceability risk. The systems are subject to regular internal and independent third party audit as verification of compliance and appropriate risk mitigation measures.

With the globalization and integration of food supply chains focused on improved quality, food safety and traceability risk, high profile food safety and fraud scandals triggering public health concern and damaging trust, governments adopting stricter and more complicated regulations, compliance risk spread across multiple jurisdictions, shifts in economic power, scientific advancements increasing one's ability to detect hazards and identify risks, advances in traceability, far reaching social media, changing food demand and growing populations and prosperity, world-class food companies must establish internal standards that are far more stringent than those required by law. At Richardson, instead of merely complying with regulatory food safety requirements, we strive for exceptional quality and food safety that distinguishes us from our competitors and builds consumer trust and brand loyalty.

Resistance Breeding A

S-17

FHB in Brazil - challenges, successes and future work

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Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is one of the most devastating plant diseases in southern Brazil. Within this region, Passo Fundo is considered a FHB hotspot due to its environmental conditions, which favor the occurrence of the disease almost every year. Over the years, many Brazilian breeding programs have used conventional breeding techniques to improve the resistance using Frontana, Sumai#3, NyuBai, Nobeokabozu, Glenn and other known sources of resistance. Through these methods, an adequate level of resistance has been obtained. With the advent of molecular markers we came to learn that this level of resistance was achieved mostly without the introduction of the main genes and QTL present in the Asiatic sources (*Fhb 1* and other *Fhb* known genes), which must have been lost in the selection process. New legislation has pressed breeders for higher levels of resistance to *Fhb* and lower deoxynivalenol (DON) levels. We report the results of our effort to use marker assisted selection (MAS) to introduce Sumai#3 genes to elite lines of our program in order to combine the “native” resistance (possibly based in Frontana Type I QTL) with the new genes. We have successfully achieved good resistance but have not yet been able to combine that with all the traits needed to release a successful cultivar. We report on our progress and future efforts planned.

S-18

Breeding for wheat germplasm with enhanced FHB resistance and reduced DON content at CIMMYT

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Breeding for wheat germplasm resistant to FHB and DON is an indispensable component of wheat improvement at CIMMYT. Newly developed advanced breeding lines are screened for FHB resistance and the results are used as an important criterion for selection. Promising lines, largely selected from breeding materials, as well as from germplasm bank collection and other sources, are selected for the development of FHB screening nursery (FHBSN), members of which must have gone through at least 3 years of FHB evaluation before being distributed globally for the utilization of national breeding programs. A haplotyping system is used to assess the presence of well-known FHB QTL, and the results are also considered while making selections. Conducting genetic and molecular analysis is another important component aiming at the understanding of FHB resistance QTL and the identification and utilization of molecular markers. Recent QTL mapping studies and haplotyping works indicate the importance of QTL such as 2DLc and 4BS, as well as a lack of strong Type II resistance genes like *Fhb1*. The latter problem is mainly caused by the repulsive linkage between *Fhb1* and *Sr2* that is a durable stem rust resistant gene and has been widely utilized in CIMMYT germplasm. Efforts have been successful to overcome this problem with the development and utilization of germplasm with the two genes in coupling linkage. Greenhouse screening for Type II resistance has also been paid more attention in order to improve the Type II resistance level in CIMMYT germplasm.

S-19

FHB resistance breeding in durum wheat: Progress and perspectives

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Fusarium head blight (FHB) in durum wheat causes severe economic losses and the associated mycotoxin is a food and feed safety issue. Consequently breeding for resistance to FHB in durum wheat is of highest priority in Canada. Enhancement of FHB resistance in durum wheat has been made, with progress from predominantly susceptible to a proportion of lines with intermediate resistance in advanced breeding materials. Improving resistance is slow because of lack of variation in elite germplasm, and the trait is controlled by many minor effect genes highly affected by environmental conditions. The challenge is compounded by the requirement to recombine FHB resistance with other important traits such as leaf spotting disease resistance or midge resistance while maintaining the complex grain quality and agronomic package of Canada Western Amber Durum. Recent initiatives in FHB resistance breeding include increasing the number and size of FHB nurseries, selection for FHB response at earlier generations, use of recurrent selection, crossing with resistant hexaploid lines, infusion of more resistant exotic germplasm and wild relatives, use of DNA markers, and adoption of genomic selection. Recently developed genomic tools are being used with phenotyping bi-parental, association mapping, and nested association mapping panels to identify new quantitative trait loci (QTL) and to develop high throughput single nucleotide polymorphism (SNP) markers. Ultimately discoveries from this research are being funnelled towards large-scale marker assisted selection (MAS) in our breeding programs. We anticipate the application of sustained and integrated approaches in FHB resistance breeding will lead to durum varieties with resistance enhanced beyond the current intermediate level.

S-20

Breeding for enhanced fusarium head blight resistance in Canadian bread wheat

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Canadian bread wheat is well known for its high quality, high protein content and its versatility to make pan bread, noodles, and pasta in different parts of the world. Canada ranks sixth in the world for hard red spring wheat production, the vast majority of which is produced in western Canada. In 2015, Canada produced approximately 25 million metric tonnes of wheat, of which 17 million metric tonnes (68%) were exported contributing about \$6.2 billion dollars to the Canadian economy. Nearly 96% of the wheat is grown in the prairie provinces of Alberta, Saskatchewan, and Manitoba. Due to warmer summers and higher rainfall, the eastern Prairies have some of the highest wheat grain yields but also experience major wheat diseases. Fusarium head blight (FHB), caused by *Fusarium graminearum*, is one of the most devastating diseases of wheat causing major yield losses, grade reduction, and grain toxicity. A severe outbreak in Manitoba in 1993-94 brought the problem to prominence for the Canadian Prairies. In the following decade, *F. graminearum* slowly spread across Saskatchewan and into southern Alberta. Currently, losses to the wheat industry in Canada due to FHB range from \$50 million to \$300 million annually. Widespread cultivation of susceptible wheat varieties, which also act as an overwintering source of inoculum, is considered one of the main reasons of FHB disease outbreaks. The presentation will discuss the development of high yielding wheat varieties with increased tolerance to FHB, which would have greater acceptance by Canadian farmers, mitigate disease outbreaks, and reduce economic losses.

Resistance Breeding B

S-21

Breeding winter wheat for fusarium head blight resistance - Challenges and progress

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Fusarium graminearum (Schwabe) (FG) is the principal cause of Fusarium head blight (FHB), one of the most serious diseases of wheat. Deoxynivalenol (DON) is the most important mycotoxin produced by FG. Winter wheat is mainly grown in eastern Canada where development of wheat resistant to FHB, without significant yield and quality penalties, is important. Conventional breeding, double haploid method, and marker-assisted selection are used in our program. By using exotic sources we found that breeding lines grouped in the 3B QTL class had the lowest FHB index, DON content and FDK level and did not have a significantly lower yield or protein content compared to the lines grouped in other QTL classes. We recently found QTL for FHB resistance on chromosomes 2D, 4B and 4D, in a population with native source of resistance (soft red winter wheat 'Vienna'). Phenotyping of additional winter wheat populations is in progress. Genotyping and QTL analysis will be performed using Illumina Infinium 90K BeadChip platform. All wheat commercially grown in Ontario is entered in the Performance Trial and tested in agronomy trials with and without fungicide application and for FHB resistance in three nurseries spray inoculated with FG. 'Marker', a soft red winter wheat developed by our breeding program is moderately resistant to FHB and is used as a check in Ontario when testing lines for FHB resistance. 'UGRC Ring' is our new high yielding winter wheat assigned to moderately susceptible category, with respect to FHB resistance, and will be grown in Ontario and Quebec.

S-22

Improving FHB resistance in Canadian barley: Recent progress and future prospects

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Improving fusarium head blight (FHB) resistance incited by *Fusarium graminearum* Schwabe is an important objective for most barley (*Hordeum vulgare* L.) breeding programs in Canada, but incorporating this trait into a commercially acceptable package has been difficult. This presentation will highlight progress made over the past 5 years in Canadian barley and discuss future prospects for improvement including new methodology and genetic research. It will also note recent changes in collaborators, nurseries and funding. AAC Connect two-row malting barley developed by Agriculture and Agri-Food Canada (AAFC) Brandon and registered in 2016 has a desirable combination of agronomic, malting quality, and disease resistance traits including somewhat lower deoxynivalenol (DON) accumulation which continues to be the critical trait to measure in barley for FHB resistance. Resistance in AAC Connect traces back to the exotic two-row Chinese accession Harbin. The fate of another two-row malting line TR10214 with this resistance, and previously supported for registration recommendation, is uncertain. Also recently supported for registration recommendation in western Canada was TR13609 two-row malting barley from Field Crop Development Centre, Alberta Agriculture and Forestry, Lacombe. Most of the breeding programs have promising lines being evaluated in registration tests. In eastern Canada, AAC Starbuck two-row hulless feed and AAC Azimuth six-row hulless feed cultivars with lower DON accumulation have recently been released by AAFC Ottawa. Although good progress has been made, new genetic research and DON testing methods may hasten the release of barley cultivars that combine lower DON accumulation in a package desired by industry.

S-23

Fusarium head blight of oat --- Progress in dealing with a sly foe

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The presence of fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe and other *Fusarium* spp., was reported in producer seed lots from Manitoba and Saskatchewan as early as 1993-1994 by Clear and coworkers. During the '1st Canadian Workshop on FHB' in 1999, several research needs related to oat were identified, as a result of a lack of distinctive visual symptoms of infection in the crop, and that toxin levels were reduced through oat processing. Systematic surveys for FHB in commercial oat crops were begun in 2002. Variety performance trials were initiated with commercially available cultivars for determination of FHB reactions. Cultivars, breeding lines, and exotic accessions obtained from gene banks were evaluated for FHB reaction through the detection of causal species and the presence of deoxynivalenol (DON). Corn-spawn inoculated screening nurseries were established at Portage la Prairie, MB in 2003 and at Glenlea, MB in 2005. Crosses were initiated in 2007 between adapted lines and cultivars and the identified accessions with better FHB resistance. Mapping populations were developed in anticipation of marker development. This presentation will discuss the progress that has been made to improve FHB resistance in oats, but much more research is needed to overcome this sly foe.

S-24

proWeizen – the German wheat research and breeding alliance

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Wheat is one of the most important crops and Germany is an important wheat producer. In Germany, 16 breeding companies are running independent wheat breeding programmes.

The German Wheat Research and Breeding Alliance was founded in 2012 by German wheat breeders to combine scientific excellence in wheat research and breeding expertise in Germany. As a public-private partnership, the proWeizen alliance acts to foster wheat breeding and research on a national and international level as well as a platform for communication and coordination. The proWeizen platform is equally open to scientists and companies working in wheat breeding and research.

Currently, 11 research projects, funded by the German Federal Ministry of Food and Agriculture (BMEL) as well as the German Federal Ministry of Education and Research (BMBF), are run within the proWeizen alliance and focus on breeding for yield increase and stability, better adaptation to environmental stresses and utilization of heterosis. In these projects, German universities and research institutes are working in close collaboration with wheat breeders who are vital partners and plan to implement project results in their future breeding programmes.

In addition to support in project management and coordination, proWeizen liaises with wheat researchers and breeders and participates in wheat research and breeding on national and international levels, respectively. proWeizen also helps with mobilizing funding opportunities.

S-25

Identification of genomic regions associated with resistance to fusarium head blight, leaf rust and stem rust in winter wheat

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Fusarium head blight (FHB) is the most destructive wheat disease, mainly caused by *Fusarium graminearum* (FG). Deoxynivalenol (DON) is a mycotoxin produced by FG. Leaf rust and stem rust are also destructive wheat diseases throughout wheat-growing regions. Breeding resistant cultivars is considered the most effective, economic, and environmental way to control these diseases. In addition to exotic sources of resistance, native resistance sources are required in winter wheat breeding programs. Vienna has been grown in Ontario since 2002 and has a moderate resistance to FHB, being a potential native resistance source. The objective of this study was to map loci associated with FHB, leaf rust and stem rust in a doubled-haploid (DH) population derived from the cross of two Canadian winter cultivars, Vienna and Pioneer-25R47. DArT markers were used to generate a genetic map and QTL analyses were performed evaluating 102 DH lines for FHB severity, incidence, index, DON accumulation, leaf rust and stem rust severity and plant height in three trials in Ontario, Canada (Centralia 2011 and Ridgetown 2011 and 2012). Significant QTL for FHB resistance were found on chromosomes 2D, 4B and 4D. The phenotypic explanations for these QTL were 9.4, 10.5 and 15.4%. The FHB QTL on 4B and 4D were co-localized with QTL for plant height. QTL for both leaf and stem rust resistance were identified on 1B. The QTL for FHB, leaf rust and stem rust resistance are valuable sources of resistance to be used in marker assisted selection for development of new winter wheat cultivars.

S-26

FHB can be defeated by a systemic approach

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Based on registration trial data, to create agronomic wheat with high FHB resistance, the systemic approach (Comeau *et al.* 2010) was significantly more successful than other approaches used by other breeding programs, with p -levels from $p < 0.05$ to $p < 0.00006$ for many key traits. Complex crosses were selected with complex stress for a few generations (F_1 to F_5 - F_7). Stresses included BYDV, *Fusarium* and rust. Resistant germplasm was shared with public and private breeding projects. Breeders used it in crosses and also isolated candidate lines for cultivar registration directly from the systemic germplasm. For disease resistance traits, and especially for FHB, the systemic-derived lines significantly surpassed other candidate lines. The systemic approach led to more protein, and sometimes, better yield potential. Some systemic lines are high yielders, and others, low yielders. The systemic approach slightly reduced the undesirable correlation of high protein with low yield. Putting high FHB resistance in a short statured plant remains a challenge; a compromise with adequate lodging resistance is possible. Other methods exist to create FHB resistant lines, but so far the systemic way seems the best. Breeders should pay more attention to those ideas, in which focus is on globally better phenotypes rather than on specific genes. The method is adaptable to other crops.

Reference: Comeau A, et al. 2010. Systemic heuristic approaches guide the interaction of enhanced genetic diversity and complex stresses to generate better wheat germplasm faster and at lower cost. In: Kovalchuk I., Kovalchuk O. Genome Instability and Transgenerational Effects. Nova Sc. Publ.

Epidemiology & Disease Management

S-27

Managing fusarium head blight: Successes and challenges

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Fusarium head blight (FHB) re-emerged with devastating epidemics in the early 1990's and has subsequently proven to be a chronic problem for wheat and barley production throughout much of the United States. A coordinated research effort undertaken by the US Wheat and Barley Scab Initiative (USWBSI), the scope of which is unprecedented in the United States, has aimed to address the challenges posed to small grain production and utilization by FHB. Significant progress has been made in developing and releasing varieties with improved genetic resistance, identifying and utilizing fungicides and improved application technologies, and developing and deploying disease prediction tools. Along with an improved understanding of the impact of crop rotation and tillage on FHB risk, these tools have allowed us to develop best management practices for FHB that are effective in reducing the risk of FHB development and the contamination of harvested grain with *Fusarium* mycotoxins. Despite the considerable success of the USWBSI's research effort, FHB remains a formidable challenge to researchers, producers and end users; resistance QTL are limiting in most grain classes, being both scarce and/or only partially effective; phenotyping and genotyping is resource intensive; chemistries are generally only moderately effective and forecasting tools do not provide a definitive decision aid for growers. It appears that the effective management of FHB will rely on the integrated use of multiple disease control strategies and the diligence of the research community to transfer research outcomes to producers and grain processors.

S-28

Cropping factors: The key for sustainable mycotoxin management in cereals

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To develop recommendations to avoid *Fusarium* mycotoxins' contamination, extensive surveys were conducted. For wheat, harvest samples (n=686) and information on cropping measures were collected from Swiss growers (2007-2014). Grains were examined for *Fusarium* species incidence, fungal DNA, chemotype and mycotoxin content. *Fusarium graminearum* (FG; ratio of 65%) and *F. poae* (FP; 21%) were dominant. The mean deoxynivalenol (DON) content was 606 ppb and 11% of all samples exceeded the European limit for unprocessed cereals (1,250 ppb). Pre-crop maize combined with conservation tillage or ploughing resulted in an average DON content of 2,030 or 310 ppb, respectively, whereas other pre-crops led to average contents of 460 and 220 ppb, respectively. Samples from organic farms had considerably lower FG incidence. Species incidence, qPCR and chemotype data revealed that nivalenol was produced by FG, FP and/or *F. crookwellense*. To avoid exceeding the DON-limit, growers are supported by FusaProg, a forecasting system employing plot-specific cropping factors, growth stage and weather data. The barley survey (2013-2014) showed similar patterns as in wheat. In oats (2013-2015), however, FP was the most dominant species and T-2/HT-2 was detected in 91% of all samples. Analyses about key cropping factors are in progress. For growers with a maize-wheat rotation, supplementary strategies are needed. Antagonists applied during maize harvest could be promising. Under controlled environment, *Clonostachys rosea* completely suppressed the sexual FG-reproduction on crop residues. Within the European project MycoKey, different *Clonostachys* formulations are examined to ensure competitiveness under field conditions. Inter-/cover crops might reduce FG inoculum through physical barriers or antifungal properties. Preliminary results from these experiments will be presented. Integration of various preventive measures could contribute to sustainable cropping systems with reduced mycotoxin risks.

S-29

DONguard: suppression to control through microfloral shift and IPM

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The utility of DONguard™, *Clonostachys rosea* strain ACM941 for the suppression and control of fusarium head blight, is dependent on timing, adjuvant load and rate of conidia applied, colonization of the seed head of wheat, barley and other cereals and the colonization of the leaves. Colonization of leaf and stem tissue at the time of application to the head will prevent the perithecial stage emerging from the residue. DONguard effectively mycoparasitizes fusarium head blight. Application timing at about Zadoks 50 (whorl opening-early heading) is the best timing for colonization of wheat and barley. *Clonostachys rosea* needs time to colonize ahead of the disease challenge. Tank mixing with several fungicides allows an IPM program.

S-30

Metabolomics analysis of the effect of elevated CO₂ on wheat resistance to fusarium head blight

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Climate change is expected to intensify fusarium head blight (FHB) contamination of wheat and increase the associated risk of mycotoxin contamination in food and feed. Rising CO₂ levels are part of climate change with still unknown effects on natural wheat resistance mechanisms against *Fusarium graminearum*, the primary etiological agent of FHB. In this study the defence response of wheat plants grown at ambient (400 ppm) CO₂ and elevated (800 ppm) CO₂ was evaluated and compared. Both Type I, resistance to initial infection, and Type II, resistance to *Fusarium* spread throughout the wheat head, were compromised at elevated (800 ppm) CO₂ with increased pathogen biomass and trichothecene contamination. Plant and fungal metabolites play a major role in defence and virulence, and there are significant differences in the metabolic responses of resistant and susceptible plants. 1D and 2D ¹H NMR spectroscopy were performed for the metabolite assignment and quantification of metabolites in wheat grown at different CO₂ levels. These data provide putative markers which can discriminate metabolic changes that are consistent with difference in wheat susceptibility to FHB. A new method for metabolite quantification from NMR data that automatically aligns spectra of standards and samples prior to quantification utilizing multivariate linear regression optimization of spectra of assigned metabolites to samples' 1D spectra is described and used. *Fusarium* infection-induced metabolic changes in wheat grown under different conditions will be discussed in the context of metabolic network and resistance. Resistance related metabolites determined in this as well as previous, published work have been systematized for further functional analysis in the new Web database called Wheat Fungal Diseases Metabolome – WFDM.

S-31

Prevalence of *Fusarium* spp. causing head blight of spring wheat, barley and oat in Ontario during 2001-2015

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Fusarium spp. causing fusarium head blight (FHB) in cereals in Ontario was monitored in recent years. Nine species were recovered from 21,400 putatively infected kernels collected from 428 affected wheat fields in 2001-2015, 11 species from 10,100 kernels from 202 barley fields in 2005-2015, and nine species from 6,250 kernels from 125 oat fields in 2008- 2015. *Fusarium avenaceum*, *F. equiseti*, *F. graminearum*, *F. poae*, and *F. sporotrichioides* were the common species, occurring in 23-68% of the infected fields and in 1.2-28.4% of the infected kernels. The remaining species including *F. acuminatum*, *F. oxysporum*, *F. culmorum*, *F. solani*, *F. crookwellense*, *F. verticillioides*, and *F. tricinctum* occurred in <3% of the fields and in ≤0.1% of the kernels. In wheat, *F. graminearum* was predominant in all the surveyed years, which occurred in 91% of fields and in 63.1% of kernels, and represented 89% of the pathogen population. In barley, *F. graminearum* and *F. poae* were equally dominant, occurring in 63% and 75% of fields, 17.7% and 14.9% of kernels, and representing 38% and 43% of the pathogen population, respectively. In oat, *F. poae* was predominant in all the years, which occurred in 94.8% of fields and 24.9% of kernels, and represented 64% of the pathogen population. There were no significant differences in the frequency of isolation, except for *F. graminearum* which increased by 33.5% in wheat and by 2.2 fold in barley in the FHB epidemic years compared with the non-epidemic years, suggesting that *F. graminearum* was responsible for the FHB epidemics in Ontario.

POSTER ABSTRACTS

1. Cereal Genomics and Genetics

P-01

Two disease susceptibility genes for FHB in wheat

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Although several genes that affect wheat resistance against the fungal pathogen *Fusarium graminearum* have been characterized, the identification of wheat disease susceptibility factors that facilitate fungal infection and support compatibility remains limited. *Fusarium* produces mycotoxins that induce susceptibility in wheat lines harboring corresponding toxin sensitivity genes. We identified two genes, an ABC transporter (unigene 149211) and a transcription factor NFXL1, that are directly induced to a high level by treatment with the toxin deoxynivalenol (DON). There is a correlation between the expression pattern of both genes and the susceptibility of the wheat genotypes to FHB. Both genes are preferentially induced by *F. graminearum* infection in FHB-susceptible wheat cultivars relative to FHB-moderately resistant wheat cultivars. When both genes were partially silenced in the FHB-susceptible wheat cv. Roblin by using a transient viral assay (VIGS), plants exhibited some reduction in both disease spread and head bleaching. These results indicate that two DON-induced genes, an ABC transporter and the transcription factor, NFXL1, play some role in disease susceptibility to FHB in wheat.

P-02

Molecular mapping of QTL for fusarium head blight and fusarium damaged kernel resistance in spring wheat

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Fusarium damaged kernels (FDK), caused by *Fusarium graminearum*, causes downgrading and reduced quality in wheat. This study was conducted to identify DNA markers for fusarium head blight (FHB) and FDK resistance in adapted spring wheat germplasm. From a cross between moderately resistant Carberry and moderately susceptible AC Cadillac, 774 doubled haploid lines were evaluated for response to FHB in nurseries near Morden and Brandon, MB. From the continuous distributions of disease incidence (Type I resistance) and severity (Type II resistance), a 200 line subset of field resistant and susceptible phenotypes were evaluated for FDK (Type IV resistance). A linkage map of 2408 SNP (Infinium iSelect 90k SNP wheat array) and four microsatellite markers, was used to detect six significant Type I resistance QTL in both locations, eight significant Type II QTL in Brandon and five in Morden, as well as five significant Type IV QTL in Morden. Carberry carried favourable alleles for Type I, II and IV resistance on chromosomes 5A and 3B, Type I and II resistance on chromosome 7D and only Type II resistance on chromosome 3A. AC Cadillac contributed favourable alleles for Type I, II and IV resistance on chromosomes 3A, 2B and 4B, Type I and II on chromosomes 4A and 6B and only Type I resistance on 5A. These results indicate that the genomic regions contributing to reduced incidence and severity also contributed to a lower level of FDK. The QTL markers could be used to accumulate resistance loci in adapted wheat cultivars to lower FDK and improve quality.

P-03

Modulation of hormone signalling may not be the primary mechanism by which abscisic acid and gibberellic acid modify *F. graminearum* infection in wheat

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We recently demonstrated that the co-application of abscisic acid (ABA) or gibberellic acid (GA) to the wheat head, with *F. graminearum* inoculation, increased and decreased FHB spread respectively (Buhrow et al., 2016). Following up on this finding, an extensive RNASeq analysis of the transcriptional effects of these hormone treatments on wheat plants, 24 hours after co-application with pathogen, was carried out. While the bulk of the data is still under analysis, here select preliminary results pertaining to the effects of ABA and GA treatments on their own biosynthetic pathways, as well as hormone signalling pathways more broadly, are discussed. *F. graminearum* inoculation alone had mixed effects on hormone biosynthesis, down regulating some of the more upstream components of the pathways, but upregulating some of the downstream genes involved in degradation or storage product formation. Interestingly, while ABA elicited the expected induction of its own biosynthesis (rescuing some of the early pathway repression induced by the pathogen), GA was found to repress its own early stage biosynthesis even more. Neither ABA nor GA co-application affected the expression of the other's biosynthetic pathway. On the side of hormone signalling, the pathogen-alone elicited a variety of mixed responses on ABA, auxin, cytokinin, and salicylic acid (SA) pathways. As well, de-repression of the jasmonic acid (JA) signal pathway, induction of ethylene signalling genes and repression of brassinosteroid signalling genes are highlighted. The impact of co-application of ABA on this profile was limited to mixed modification of the responses on ABA signalling genes, and some reversal of the responses in the auxin and brassinosteroid pathways. The impact of GA was even less significant, leading to only a slight induction of a negative regulator of the auxin pathway. Overall, these data suggest that the effect of these two hormones on pathogen susceptibility and resistance respectively may NOT be linked primarily to modulation of plant hormone responses. Investigations are ongoing.

P-04

Host-induced silencing of *Fusarium culmorum* genes protects wheat from infection

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Plants producing antisense or double-stranded RNA molecules that target specific genes of eukaryotic pests or pathogens can become protected from their attack. This beneficial effect was also reported for plant-fungus interactions and is believed to reflect uptake of the RNAs by the fungus via a yet unknown mechanism, followed by target-gene silencing. Here we report that wheat plants pre-infected with barley stripe mosaic virus (BSMV) strains containing antisense sequences against target genes of the fusarium head blight (FHB) fungus *F. culmorum* caused a reduction of corresponding transcript levels in the pathogen and reduced disease symptoms. Stable transgenic wheat plants carrying an RNAi hairpin construct against the β -1,3-glucan synthase gene *FcGls1* of *F. culmorum* or a triple combination of *FcGls1* with two additional, pre-tested target genes also showed enhanced FHB resistance in leaf- and spike inoculation assays under greenhouse- and near-field conditions, respectively. Microscopic evaluation of *F. culmorum* development in plants transiently or stably expressing *FcGls1*-silencing constructs revealed aberrant, swollen fungal hyphae indicating severe hyphal cell wall defects. The results propose HIGS as a plant protection approach that may also be applicable to highly FHB-susceptible wheat genotypes. To better understand whether HIGS is a natural phenomenon, small RNAs from *F. graminearum* infected barley have been sequenced and functional analysis of potential HIGS targets in *F. culmorum* is in progress.

P-05

Mapping fusarium head blight resistance and DON related QTLs in Triticale

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A doubled haploid (DH) population from the cross TMP16315/AC Ultima was evaluated for fusarium head blight (FHB) Type-I (disease incidence; DI), Type-II (disease severity; DS) and Type-III (DON content) resistance at Beloeil and Ottawa in 2011. DI and DS were used to estimate FHB visual rating index (VRI) at both locations. High-throughput genotyping of the DH lines was performed with the Wheat 90K Infinium iSelect SNP Assay and the Rye 10K SNP Assay. A total of 5274 high quality polymorphic SNPs were identified and used for linkage mapping. These SNPs were mapped on all 21 Triticale chromosomes with a marker density of 2.09 SNP/cM. The high-density genetic map along with phenotypic data (DI, DS, VRI and DON content) were used for QTL mapping which identified a total of 26 QTLs including QTLs with main (additive effect; 21), epistatic (2) and additive × environment (A*E; 3) interaction effects. These QTLs mapped on 9 different chromosomes which belong to all three genomes. However, the most pronounced QTL localized to chromosomes 4R and 5R. *QFhb.lrc-5R* regulates expression of DI, DS and VRI, and *QFhb.lrc-4R* regulates VRI and DON expression. About two-thirds of QTLs for improved FHB response originated from TMP16315. These results suggest that pyramiding of these QTLs along with others can be useful for FHB tolerant and low DON content for triticale cultivar development.

P-06

Molecular characterization of *Fusarium* resistance from *Elymus repens* introgressed into bread wheat

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A cross was made of *Elymus repens* into wheat cultivar Crocus and BC progeny were advanced to BC1 F7 by single seed descent. Sixteen lines were selected based on agronomic performance and evaluated in a FHB epiphytotic nursery. Eight lines with resistance to FHB were selected. GISH analysis revealed many complex chromosome numbers and recombination in the derived lines. The least complex recombinant line was P1142-3-1 with 42 chromosomes with one pair of chromosomes showing telomeric recombinants on both arms. This wheat chromosome was identified as 3D by applying several SSR markers from every arm of every wheat linkage group and noting those that provided no signal. Lines with single telomeric recombinants were produced by additional backcrosses to Crocus and inoculated with FHB spores. It was found that the resistance was contributed by the recombinant on the long arm of chromosome 3D. These lines have minimal linkage drag and should be amenable to applications in breeding for disease resistance.

P-07

Transfer of FHB resistance from *Thinopyrum elongatum* to bread wheat

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Germplasm with good FHB resistance will normally contain a number of major resistance QTL plus a number of minor QTL all spread over the entire genome. It was thus unusual to find an aneuploid with a single chromosome (7E) introgressed from *Thinopyrum elongatum* (2x) to have high level of FHB resistance (9.6% infected florets following point inoculation compared to the parental cultivar at 57.5%). The ph1b mutant was used to induce recombination between chromosome 7E and a homoeologous wheat chromosome. The *Ph* mutant was crossed and backcrossed once to the three substitution lines 7E (7A), 7E (7B), 7E (7D) giving 556 BC1 seeds. The seeds were screened with marker PSR 574, specific for Ph1b, and 43% of the BC1 progeny were found to be homozygous recessive for Ph1b. Those plants were inoculated with FHB spores and meiosis studied in them. Progeny from resistant plants that showed complete chromosome pairing at meiosis were further analyzed. In excess of 500 BC1F1-F5 recombinants were screened with 7E-specific markers (Crop Sci 56:354-364, 2016; Genes Genom 34:67-75, 2012) and point inoculated with FHB spores, to isolate recombinants with FHB resistance and minimal introgression of 7E chromatin. The most promising recombinants were screened with markers from wheat chromosomes 7A, 7B and 7D to determine where the introgressions were located. Preliminary results indicate that the introgressions took place at marker WMC 150 on chromosome 7D, marker WMC10 on chromosome 7B and marker gwm554 on chromosome 7A. GISH analysis confirmed the locations of some of the introgressions.

P-08

Pyramiding FHB resistance QTL in spring wheat

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We were interested in establishing pyramids of several FHB resistance genes to increase the overall level of resistance in spring wheat. The genes on chromosomes 3BS (Fhb1), 3Bm, 4B were well characterized in previous studies (Somers *et al.* 2005, TAG 111:1623-1631). Two QTL with R^2 values of 20+32% were mapped on chromosome 5AS and 5AL of wheat accessions PI277012 (Chu *et al.* 2011, TAG 123:1107- 1119). An F5 population of 80 lines was produced from the crossing of line Hc374 (3Bs, 3Bm, 4B) X PI277012 (5As, 5AL). The population was phenotyped by point inoculation in a growth chamber using standard techniques and genotyping was carried out by using 2SSR markers for each QTL. A total of 16 genotypes were recognized from markers analysis. As expected, the lowest FHB scores (% infected florets) were observed on genotypes with all four QTL. Those values were 15% infection compared to null genotypes at 60%. The resistance in other pyramids was dependant on numbers of QTL involved. The absence of the QTL on chromosome arm 3BS had the largest decrease in FHB resistance. The QTL on chromosome 4B also had major effects on the expression of resistance as found in related studies. This study serves as yet another example of the advantage of pyramiding well characterized FHB QTL to enhance resistance in wheat.

P-09

Carbohydrate fractionation and profiles in the rachis of wheat varieties susceptible and resistant to *Fusarium graminearum*

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Fusarium head blight (FHB) is a serious disease of wheat caused by *Fusarium graminearum* which affects both yield and quality of wheat. Plant resistance to pathogens is comprised of a complex network of constitutive and inducible defensive barriers. The cell wall of the wheat rachis is the first major barrier that pathogens must overcome to successfully colonize plant tissues. Therefore, the main objective of this study was to determine the cell wall composition of the rachis in two wheat varieties: Chinese Spring (CS) a susceptible variety, and CS-7EL, an addition line of Chinese Spring that carries a fragment of the long arm of chromosome 7E of *Thinopyrum elongatum*, which confers resistance to FHB. Both lines were point inoculated at anthesis, and the heads collected at day 4. Sequential gravimetric analysis was conducted to fractionate the rachis, and the carbohydrate profiles of free sugar, soluble polysaccharides, and insoluble polysaccharides were evaluated by HPLC. Cellulose, hemicellulose, and lignin were the three major structural components of the rachis with $33.93 \pm 1.6 \%$, $29.62 \pm 1.4 \%$, and $12.87 \pm 1.50 \%$ (w/w), respectively. The main sugars in the ethanol fraction for both varieties were sucrose, glucose, and fructose, while the soluble and insoluble polysaccharides comprised mainly glucose followed by xylose and small amounts of arabinose. Although the glucose level in the insoluble polysaccharide fraction showed no significant difference in the control treatment for both varieties, the fungus inoculated treatment showed significantly higher glucose for CS-7EL compared to CS. The same trend was obtained for sucrose content in the ethanol fraction. However, glucose and fructose in CS-7EL were higher than in CS, both in control and inoculated treatments. These results suggest that sucrose in the free form is the preferred substrate for *Fusarium*. Also, the cellulose/hemicellulose polysaccharides of the rachis in CS are degraded relatively easily by the fungus, but the cell wall presents a much stronger barrier in CS-7EL.

P-10

Identification of genes associated with the 2DL QTL locus for fusarium head blight resistance

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The 2DL QTL from Wuhan 1 is a moderate resistance locus for *Fusarium* head blight (*FHB*), which has potential to improve the *FHB* resistance of bread wheat, and confer effective and durable resistance to novel wheat breeding lines. To identify genes associated with the 2DL QTL resistance locus, differentially expressed genes were identified by comparing a line carrying the 2DL QTL with a null sister line. Fourteen differentially expressed genes that were located on the 2DL chromosome arm, based on sequence information from the wheat genome, were characterized further by RT-qPCR; five of them were identified as having a similar differential expression pattern in 3 pairs of +/- 2DL lines. Another gene, called UN25696 and identified in previous expression work using microarray, was also confirmed to have a differential expression pattern in the 3 pairs of lines. Expression of the six candidate genes was then characterized in 78 lines of the double haploid mapping population derived from the cross Wuhan 1 x NuyBai, the population where the 2DL QTL was first identified. The expression QTL for genes UN25696, Traes_2DL_179570792 and Traes_2DL_89A313AC3 overlapped with the mapping interval for the 2DL QTL; however that of UN25696 was centered very close to the peak of the 2DL QTL. Our results suggest that UN25696, and possibly all three genes, contribute to the *FHB* resistance associated with the 2DL QTL.

P-11

Combining QTL mapping with transcriptomics to identify candidate genes for *Fusarium* resistance in maize

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Despite the economic importance of the hemibiotrophic pathogen *Fusarium graminearum*, it has been challenging to identify genes conferring resistance in maize because the trait is quite complex and highly influenced by the environment. In the current study, we attempted to characterize defence responses in two maize inbred lines with different levels of resistance to the pathogen by combining information from differential gene expression analysis and quantitative trait loci mapping of resistance. Gene transcripts responding to fungal infection were captured using RNA-seq profiling of mock and fungal inoculated maize ears, and gene ontology terms associated with up-regulated gene transcripts were determined for each inbred. More genes were up regulated in the susceptible inbred relative to the resistant inbred, many of which are associated with oxidation-reduction processes potentially causing earlier programmed cell death in the susceptible inbred. Although the hypersensitive response has been effective in controlling biotrophic pathogens, hemibiotrophs can use it to their advantage to interfere with other forms of host resistance mechanisms. We have identified differentially expressed genes located within quantitative trait loci regions of gibberella ear rot resistance previously identified using a large recombinant inbred line population; these candidate genes are involved in the biosynthesis of anti-microbial proteins and/or phytoalexins, membrane proteins (integral as well as transporters) and enzymes responsible for detoxification of xenobiotics.

P-12

QTL Mapping of fusarium head blight resistance in an elite winter wheat doubled haploid population

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In Canada, *Fusarium graminearum* is the primary causal agent of fusarium head blight (FHB) in wheat, resulting in yield and end-use quality losses. Resistance to FHB is complex and often involves multiple genes with relatively small effects. As a result, the breeding strategy is to combine different types of FHB resistance into a single genotype. The breeding line 32C*17 showed strong FHB resistance under severe disease pressure in both Canada and Germany. Two hundred doubled haploid lines were made from a cross between 32C*17 and a moderately resistant line (18I*45). The 90K wheat Illumina Infinium iSelect single nucleotide polymorphism array was used to genotype this population. Replicated field trials inoculated with a *F. graminearum* macroconidial suspension were conducted at three locations in 2015 and two locations in 2016. Dual floret inoculations were performed in the greenhouse in a replicated trial. Inoculum used in all trials was a mixture of two isolates of 3-Acetyldeoxynivalenol chemotype producers and two isolates of 15-acetyldeoxynivalenol chemotype producers. Plant height, disease incidence, disease severity and FHB Index were measured in the FHB field nurseries. Fusarium damaged kernels and deoxynivalenol (DON) contents were determined from field nursery harvested grain samples. Greenhouse disease severity data was also estimated. Transgressive segregation was observed for all FHB measured traits. Multiple quantitative trait loci (QTLs) were detected including a QTL for plant height. Increasing the plant heights may contribute strong Type I resistance resulting in low FHB Index, FDK and DON. Further characterization of FHB resistance in this population is ongoing.

P-13

Response to infection by *Fusarium graminearum* in the rachis of a resistant and a susceptible barley cultivar

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To evaluate the response of resistant and susceptible barley cultivars (cvs) to *Fusarium* head blight (FHB), structural and chemical changes were investigated in the rachis of Chevron, a resistant cv, and Chapais, a susceptible cv, after inoculation with *Fusarium graminearum*. Microscopy of cross sections of the rachis showed differences in severity of infection, with more fungus being visible in the vascular bundles of Chapais than in Chevron 5 days post-inoculation. In addition, the chemical composition of the cell walls in the vascular bundles of Chevron underwent changes, as evidenced by the loss of fluorescence in this tissue. Sequential gravimetric analysis was conducted to elucidate chemical changes in the rachis, followed by sugar profile analysis using HPLC-ELSD. The results showed that in all treatments, cellulose was the fraction with the highest content. In both cultivars, while the content of hemicellulose and cellulose decreased after inoculation with *Fusarium* compared to the mock inoculated samples, indicating some cell wall degradation by fungal enzymes, the lignin and water soluble components increased. The content of lignin in *Fusarium* inoculated samples of Chevron (23.2 ± 1.2 %, w/w dried rachis tissue) was significantly higher than in Chapais (12.2 ± 1.2 %), which could reflect cell wall fortification in Chevron as a defence against FHB. Sucrose was the predominant free sugar in the water soluble fraction of all treatments, however, the total content of free sugars (sucrose, glucose and fructose) was significantly higher in the *Fusarium* inoculated Chapais (5.3 ± 0.2 %, w/w dried rachis tissue) compared to Chevron (4.5 ± 0.2 %), suggesting greater fungal degradation of the cell wall and stored fructans in the susceptible cultivar. The sugar composition of the cellulose fraction was similar for all treatments, glucose being the main component with small amounts of xylose and fructose. However, the content of glucose was higher in the rachis of inoculated Chevron compared to Chapais, suggesting stronger cell wall structure in the resistant barley.

P-14

Identification of quantitative trait loci (QTL) associated with fusarium head blight resistance in a D8006W/ Superior winter wheat population

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Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the major diseases of wheat in North America. FHB infection reduces grain yield, affects end-use quality and accumulates mycotoxins such as deoxynivalenol (DON) in the grain. The objective of this research is to identify QTL associated with FHB resistance. A doubled haploid winter wheat population consisting of 107 lines from a cross of soft white winter wheat D8006W and Superior is being used. Evaluation for FHB reaction was performed using spray inoculation of a mixture of *F. graminearum* isolates representing two chemotypes in replicated field disease nurseries in Winnipeg, Carman, and Ridgetown in 2016. Disease incidence and severity were recorded 21 days post inoculation and FHB index was calculated. Both parental lines showed moderate reaction across all locations; D8006W had a mean FHB index of 6.2, 10.3 and 14.4 while Superior had a mean FHB index of 3.5, 8.0 and 8.3 at Winnipeg, Carman and Ridgetown, respectively. The population showed a continuous distribution pattern and transgressive segregation of progeny with average FHB index of 6.1 (range=0.3-34.5), 16.0 (range=1.6-57.2) and 12.0 (range=0-42.0) at the given three locations, respectively. Percentage Fusarium damaged kernels and DON content will be measured from collected grain samples. Genotyping of the population will be performed using the Illumina Infinium 90K BeadChip platform and QTL analysis will be performed. This experiment will be repeated in 2017. This work will identify significant QTL for FHB resistance that can be used for marker assisted selection in winter wheat breeding programs.

P-15

Differential gene expression feature extraction from FHB challenged wheat RNA-SEQ data

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In differential gene expression data analysis, one of our objectives is to identify groups of co-expressed genes from a large dataset to detect the association between a group of co-expressed genes and a phenotypic trait. This has usually been done through various clustering approaches, such as k-means and bipartition hierarchical clustering, based on particular similarity measures in the grouping process. In a differentially expressed gene dataset, the differential expression itself is an innate attribute that can be used in the feature extraction process. For example, in a FHB affected wheat differential expression gene dataset consisting of one FHB susceptible line and n FHB resistant lines, the expression of a gene in each line would have three possible behaviors, up- or down-regulated, or unchanged after Fusarium challenge. We used three numerical values to denote such behavior, i.e. 1=up, 2=down, and 0=unchanged. As a result, we have up to 3^{n+1} differential expression patterns across all $n+1$ lines. Nevertheless, the actual number of patterns will be smaller than that since not all theoretical patterns would have a gene. This presentation is to demonstrate a series of successful applications of such a feature extraction scheme in wheat gene differential expression data analysis under Canadian Wheat Alliance collaboration between NRC and AAFC.

P-17

A genome-wide association study reveals novel fusarium head blight resistance QTL in the durum breeding lines derived from the DT696 source of resistance

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Commercial durum cultivars have a low level of resistance to fusarium head blight (FHB) with devastating consequences in favourable disease environments. A Genome-Wide Association Study (GWAS), which benefits from the recombination events in diverse genotypes, is well suited for genetic dissection of QTL. GWAS was conducted on 401 breeding lines derived from the *Triticum turgidum* ssp. *durum* line DT696, an adapted source of FHB resistance in Canada. The analysis was conducted using 9043 Single Nucleotide Polymorphism (SNP) markers generated using the 90K iSelect high density genotyping array and phenotypic data collected for these lines since 2004 that included FHB incidence, severity, index and intensity (visual rating of plots) and developmental traits that modulate resistance including plant height, maturity and lodging. SUPER compressed mixed linear model (cMLM) controlling for population structure and relatedness was found to be the most appropriate for marker-trait association. Significant marker-trait associations were detected on chromosomes 6BS and 6BL for FHB incidence, 2B and 7B for FHB severity and on 6AL for FHB intensity. The genomic intervals with FHB QTL harboured orthologues of genes involved in plant defense responses. None of FHB resistance QTL were linked to FHB-modulating developmental traits. SNPs associated with the resistance QTL could be used for high-throughput and rapid selection in durum wheat breeding gene pyramiding programs.

P-18

NAC transcription factor and laccase gene confirms rachis resistance in wheat near isogenic line containing QTL-Fhb1

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Wheat is a staple food which provides a major source of starch and energy for millions of people. Fusarium head blight (FHB) is one of the most devastating and alarming diseases of wheat around the globe. In addition to causing loss in yield, it also reduces grain quality by contamination with mycotoxins. Among 121 quantitative trait loci (QTLs) associated with FHB resistance, QTL-Fhb1 is considered to have a major resistance effect. A fine mapped QTL-Fhb1 located within a 1.27cM interval (S/T) was sequenced to identify the candidate resistance *R* genes. Wheat near isogenic lines (NILs), derived from Sumai-3 and Thatcher cross, were sequenced based on Illumina high-seq technology to capture the genes localized within the QTL-Fhb1 region. The genes with plausible resistance functions were: TaNAC transcription factor, laccase, ubiquitin-conjugating enzyme E2 23, cytochrome P450 72A13-like, 70 kDa heat shock protein, G-type lectin S-receptor-like serine/threonine-protein kinase and calmodulin TaCaM1-3 mRNA. TaNAC and laccase coding genes were found to be polymorphic, non-functional in S-NIL. In addition, *TaNAC* regulates flavonoid biosynthesis and laccase is involved in monolignol biosynthesis. The metabolic profiling of NILs revealed high accumulation of free phenyl propanoids, phenolic glucosides, lignans and flavonoids related metabolites. Some of the resistance related metabolites such as lignans are considered to be biosynthesized by laccase which might get regulated by NAC transcription factor. Functional validation of these genes will be done through Virus Induced Gene Silencing (VIGS), for potential use in breeding.

P-19

Towards the identification of candidate gene(s) for FHB resistance on the 7EL chromosome of *Thinopyrum elongatum*: design and use of genetic markers

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Thinopyrum elongatum is a wild grass that carries genetic resistance to FHB on the long arm of its chromosome 7E (7EL). 7EL-specific markers as well as 7DL-specific markers for homoeologous wheat sequences were designed to help characterize introgressed material derived from the cross CS ph1b x CS-7E(7D), a cross that facilitated the introgression of 7E fragments into 7D. As neither wheat nor *Th. elongatum* genomes are fully sequenced, a cross-walking strategy between wheat and *Th. elongatum* draft genomic sequences was used. Twelve pairs of markers for homoeologous sequence regions of 7EL and 7DL chromosomes, six individual 7EL-specific markers and four 7DL-specific markers were successfully designed in this project. Those markers were used to characterize BC₁F₄ progeny from three families derived from the cross CS ph1b x CS-7E(7D), to help define the introgressed 7E fragments that they contain, in complement with previously published markers. Two of the families contained a smaller 7EL fragment still carrying FHB resistance, when compared with the third family. That smaller introgressed 7EL fragment replaced about half of the 7DL chromosome, based on absence of 7DL markers. The novel 7EL- and 7DL-specific markers as well as the proposed genetic order for novel and previously used markers significantly contributed to the characterization of the introgressed 7EL fragments in the 7DL chromosome.

P-20

Computational discovery of genetic markers for wheat fusarium resistance

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To investigate the mechanism of response to *Fusarium*, many studies describing the interactions between *Fusarium* and wheat compare two distinct parental lines contrasting for resistance to FHB. These studies mainly focus on identifying *Fusarium* responsive genes. Using a breeding population containing resistant and susceptible lines of wheat, we have targeted the identification of gene modules and genes that explain the phenotypic differences among susceptible and resistance lines. We have generated gene modules and performed statistical analysis to identify gene modules showing distinct transcript abundance levels among susceptible and resistant lines using the RNAseq data from *Fusarium* vs water treated samples in multiple susceptible and resistant lines. These gene modules are combined with *Fusarium* responsive genes to identify gene modules and genes that are responsible for *Fusarium* resistance differences among susceptible and resistant lines. The downstream gene annotation and pathway analysis further describe the role of these genes.

P-21

Evaluation of Genomics prediction for fusarium head blight resistance within a multiparental population

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Fusarium head blight (FHB) resistance is quantitatively inherited, controlled by multiple genes with minor effects, and highly affected by the interaction of GXE. Therefore, the evaluation FHB with conventional approaches lacks accuracy, rendering breeding for this trait time consuming and costly. The advent of cost-effective genotyping systems allows the application of genome-wide marker information to calculate genomic estimated breeding values. With this approach, selection can be made on GEBV without phenotyping, accelerating breeding for FHB resistance. GS involves predicting breeding values based on genome-wide markers using a model trained with phenotypic and genotypic data. A multi-parental population with novel FHB resistance was created with FL62R1, an Eastern Canadian spring wheat line with good FHB resistance, through crossing with two CWRS wheat varieties and the winter wheat variety, Emerson. This multi-parental population was phenotyped for FHB severity (SEV), incidence (INC), Fusarium Damaged Kernels (FDK), deoxynivalenol levels (DON), and heading date at Carman, MB, and Ottawa in 2015 and 2016. This multi-parental population was also genotyped with the 90K SNP chip. The large phenotypic and high-density marker datasets allowed us to implement the GS approach and evaluate the prediction accuracies of GS on FHB resistance component traits. For all traits, the prediction accuracies were evaluated on: 1) different GS models; 2) different marker sets; 3) size, composition, and genetics properties of training population; 4) multiple traits; 5) GXE interactions. After optimization of all these components, improvements to the accuracy predictive models were established. With improved models, moderate to high prediction accuracies were achieved for these FHB resistant traits. This research also provided the opportunity to optimize a strategy to improve the accuracies of GS.

2. Pathogen Genomics & Genetics and Population Biology

P-22

Regulation of gene expression through DNA methylation in the cereal crop pathogen *Fusarium graminearum*

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Fusarium graminearum is a fungal pathogen of cereal crops and causes fusarium head blight disease in wheat. *F. graminearum* utilizes specialized metabolites during infection, including the toxin deoxynivalenol (DON). DON is an essential specialized metabolite for *F. graminearum*, required for pathogenicity. It is produced by the *Tri* gene cluster located on chromosome 2. Two regulatory genes of this pathway are *Tri6* and *Tri10*; these genes are expressed under nutrient poor conditions, but not under nutrient rich conditions. Recent literature has indicated that epigenetics, such as histone modification, plays a key role in regulating the genes responsible for producing these secondary metabolites. We were interested to know if DNA methylation may also be responsible for regulating these genes in response to changes in environmental conditions. DNA methylation was assessed through whole genome bisulfite sequencing (WGBS). In WGBS, non-methylated cytosines are chemically converted to thymine. Methylated cytosines are protected from conversion. Methylation sites on *Tri* genes of interest were validated by methylation specific PCR. The results demonstrated a dynamic change in the methylation of specific sites in the promoter regions of the *Tri6* gene. Targeted gene deletions of two DNA methyltransferases indicated that expression of regulatory genes *Tri6* and *Tri10* is reduced by >20%. Future efforts involve characterizing DNA methyltransferase mutants, and their role in the infection process. The study of DNA methylation may help identify mechanisms of rapid gene regulation in response to changing environmental conditions in the fungal pathogen *F. graminearum*, as well as in other filamentous ascomycete fungi.

P-23

PAMP perception during *Fusarium graminearum* pathogenesis

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Resistant wheat cultivars recognize unknown signals during *Fusarium graminearum* infection to limit fungal spread to healthy spikelets. Fungal infection of a spikelet induces signaling and defense gene expression, cell wall thickening and resistance to subsequent infections in the surrounding spikelets. These responses are associated with perception of pathogen-associated molecular patterns (PAMPs) in other host-microbe interactions suggesting that similar host recognition mechanisms may influence *Fusarium* pathogenesis. To identify *Fusarium* elicitors we first determined that *Fusarium* extracts produced a dose-dependent protection against subsequent bacterial infection in *Arabidopsis*. Using both protein fractionation and purified proteins, we identified several candidate elicitor proteins which induce protection against subsequent infection. Preliminary results indicate that one elicitor in particular induces PAMP-like signaling responses including MAPK3 and MAPK6 phosphorylation within a similar time frame as other PAMPs. This elicitor may be detected extracellularly since two receptor-like kinases are required for protection and the response is BAK1/BKK1-dependent. Together, this suggests a role for PAMP perception in the *Arabidopsis-Fusarium* interaction.

P-24

Characterization of three *Fusarium graminearum* overexpression mutants and their ability to elicit host defence response in cereals

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Fusarium graminearum is the primary causal agent of fusarium head blight disease in cereals. With the objective of identifying pathogen-associated molecular patterns (PAMPs), three proteins were selected from the *F. graminearum* secretome based on their sequence homology to proteins showing pathogenicity in other plant-pathogen interactions. *F. graminearum* mutants were generated by overexpression (OX) or knockout (KO) of genes encoding two CFEM-domain containing proteins (FgCFEM-1 and -2) or cerato-platanin (FgCP). CFEM is a fungal-specific domain found in some membrane proteins and is thought to have roles in fungal pathogenesis. CP is a phytotoxic protein located in the fungal cell wall and is known to be involved in plant-pathogen interactions. Evaluation of the CFEM and CP mutants (OX and KO) and their ability to cause disease in wheat, barley and *Brachypodium* is underway. Preliminary data for the FgCFEM-1(OX) mutant, evaluated in spikes of the susceptible Canadian wheat cultivar 'Superb' and a moderately resistant double haploid wheat line GS-1- EM0040 ('CIMMYT 11'/ 'Superb'*2) shows significant reduction in aggressiveness compared with either the wild-type strain or the FgCFEM-1(KO) mutant. It is hypothesized that this *F. graminearum* protein is a PAMP, and that its overexpression leads to activation of PAMP-triggered immunity. To further assess the roles of these candidate PAMPs, the ability of purified FgCFEM-1, FgCFEM-2 and FgCP to induce host- defence responses is being evaluated using a combination of gene expression analysis, activity assays, and pathology tools.

P-25

Redox proteomics identifies redox-sensing proteins targeted by NADPH oxidase in *Fusarium graminearum*

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Fungal NADPH oxidases (NoxA and NoxB) are regulated to generate superoxide and ultimately hydrogen peroxide as an intracellular signal to target specific proteins. Susceptible sulfhydryl groups can be oxidized reversibly and their redox state can act as a molecular switch to modulate protein function. We have used two strategies to label reactive cysteines in *Fusarium graminearum*, either with biotin to facilitate affinity enrichment, or with monobromo bimanane, a fluorescent label, to facilitate their detection on 2D gels. We then used LC-MS/MS to identify proteins and their modified cysteines in comparative analyses with a wild-type strain of *F. graminearum* and a $\Delta noxAB$ deletion mutant. *F. graminearum* is a multicellular fungus that causes serious economic losses in cereal crops worldwide. The $\Delta noxAB$ mutant lacks NoxA and B and is non-pathogenic. The level of oxidized cysteines in target proteins should be lower in the mutant as it produces less H₂O₂. The labelling approaches yielded candidate redox-sensing proteins which are putative targets of redox signalling originating from NoxAB. To confirm their roles we constructed both deletion and substitution mutants (C to S) of six of these candidates and examined their phenotypes *in vitro* and *in planta*. One mutant (FGSG 10089, a protein of unknown function) is phenotypically similar to $\Delta noxAB$. The most recent results will be presented.

P-26

Salicylate degradation by the pathogen *Fusarium graminearum*

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Salicylic Acid (SA) is a major signalling hormone in plant defence. Conversely, the cereal crop pathogen *Fusarium graminearum* (*Fg*) is able to utilize SA as a sole source of carbon, and degrade it through the β -ketoadipate pathway. In this study, we selected four fungal SA responsive genes that are predicted to encode SA degrading enzymes and used a "loss of function" approach to assess them. We generated *Fg*-deletant (Δ) strains of each enzyme using a gene replacement method. Two of the selected genes shared a similar gene annotation and are predicted to encode for a salicylate-1 monooxygenase (FGSG_03657 and FGSG_09063). The two other genes were a catechol 1-2 dioxygenase (FGSG_03667) and a 2,3dihydroxybenzoic acid decarboxylase (FGSG_09061). Three isolates of each *Fg*-deletant strain were assayed for their catalytic activity. Thereafter, we focussed on the Δ FGSG_03667 strains and monitored the gene expression profiles with *in vitro* and *in planta* growth assays. FGSG_03657 and FGSG_03667 were identified as the first two key enzymatic steps of the classical SA degradation pathway. We also demonstrated by RT-qPCR analyses that expression of both genes was substrate dependent, and was regulated by feedback inhibition. Neither of those two genes, when disrupted, was shown to attenuate fungal virulence in the plant disease assay. Additional analyses revealed that FGSG_09061, predicted to catalyse a potential non-oxidative decarboxylation, also contributed to SA biodegradation. These findings will bring new basis for future research on the SA catabolism in this important pathogenic fungus.

| P-27

Potential role of small RNAs in *F. graminearum* pathogenicity

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RNA interference (RNAi) has been established as an important mechanism of gene regulation in many biological systems. Over the last decade there has been increasing evidence of its involvement in various life stages of fungal organisms. Of particular interest is RNAi involvement in the pathogenicity of fungi attacking economically important crops, such as the wheat pathogen *Fusarium graminearum*. Not only does this fungus decrease grain yield, it also produces toxic compounds as a part of its infection strategy. The toxin deoxynivalenol (DON) has been studied most extensively as a determinant of *Fusarium* virulence. The regulatory genes *Tri6* and *Tri10* that govern the production of DON have been extensively studied. We are interested in finding a relationship between RNAi pathway in *Fusarium* and its role in virulence and DON production. In our screen of wild type, *Tri6*- and *Tri10*-deficient *F. graminearum* strains grown in culture, we have identified a total of 14,484 sRNA and a subset of these are differentially expressed in response to infection and DON induction. We are currently in the process of identifying potential targets of these sRNAs.

3. Mycotoxins

P-28

Value assignment of deoxynivalenol and ochratoxin-A in a multi-mycotoxin contaminated grain product reference material

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Mycotoxins, which are produced by certain fungi, have attracted worldwide attention. They are known to have a significant impact on human and animal health and occur naturally in a wide range of agricultural commodities such as grains, spices, and dried foods. Many regulatory organizations worldwide specify maximum allowed concentrations of mycotoxins in food including deoxynivalenol (DON) and ochratoxin A (OTA). In Canada, the maximum limit for DON in wheat is 2 mg/kg for adult food and 1 mg/kg for baby food, but these levels are under review. Proposed limits for OTA in grains are 5 µg/kg. Few matrix reference materials are currently available to assist with these analyses. To provide measurement tools and promote conformity of measurement the National Research Council Canada (NRCC) is producing a mixed cereal grain material contaminated with a target level of ~5 ng/g for OTA and ~1 mg/kg for DON as a certified reference material. Precise and accurate analysis of OTA in complex matrices at low ppb levels is a challenge and the method of choice is exact matching isotope dilution mass spectrometry (IDMS). However, the high concentration of DON targeted in the flour makes the use of isotopically labeled standards prohibitively expensive therefore other methods such as standard addition and external calibration were explored. For calibration of all procedures the mass fraction of commercially sourced native OTA and DON was determined using quantitative proton NMR.

P-29

Secondary metabolite profile of Canadian NX-2 *Fusarium graminearum* chemotypes

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Fusarium graminearum isolates have traditionally been known to produce type B trichothecenes, either 15-ADON, 3-ADON or nivalenol. Recent surveys of *F. graminearum* from North America identified a “Northland” population that produced NX-2, a type A trichothecene that is not hydroxylated at the C-8 position¹. Sequence diversity of the trichothecene biosynthetic enzyme Tri8 determines whether a strain produces trichothecenes acetylated at the C-3 or C-15 positions while ~9 amino acid changes within the Tri1 protein determine the production of type A or type B trichothecenes². We had surveyed 43 *F. graminearum* strains isolated from Canadian wheat and grass samples collected in 2010 and 2011. Sequencing the *Tri8* gene of these isolates revealed that 22 possessed the 3-ADON genotype while sequencing the *Tri1* gene revealed that two showed genetic similarity to the NX-2 genotype. One NX-2 strain, DAOM242077 was from Nova Scotia while the other, DAOM250010, was from central Alberta. When cultured in two stage liquid media, the crude extract of the fungal medium showed that both isolates produced substantial quantities of NX-2 (7-hydroxy-15-deacetylcalonectrin). In addition, the minor trichothecenes detected were consistent in lacking an OH at C-8, including 7-hydroxycalonectrin, 7-hydroxyisotrichodermin and two unidentified trichothecenes. Significant quantities of culmorin, sambucinol, and some butenolide were produced, demonstrating that the biosynthetic pathways to alternate sesquiterpenes and modified trichothecenes were unaffected. When inoculated into wheat heads and subsequently analysed using DON ELISA, the de-acetylated NX (equivalent to DON) showed cross reactivity (extent of cross reactivity yet to be determined).

1. Varga et al. 2015, doi:10.1111/1462-2920.12718.
2. Kelly et al. 2016, doi:10.1016/j.fgb.2016.08.003.

P-30

Trichothecene-water interactions may offer insights into their toxicity mechanisms

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Fusarium head blight (FHB) is a devastating disease that affects the Poaceae family, decreasing grain yield and quality. FHB is caused by *Fusarium* species, which produce trichothecene mycotoxins that inhibit protein translation in plants and animals. Trichothecenes are composed of three rings: a cyclohexene (A ring) bound to a tetrahydropyran (B ring) which is bridged by a two-carbon chain at C2 and C5 forming a cyclopentyl moiety (C ring). An epoxide group, shown to be essential for toxicity, is bound to C12, which is shared by the B and C rings. Additionally, there are five positions at which functionality varies: C3, C4, C7, C8 and C15. A small number of structure-activity relationship studies have been carried out to gain insights into how different substitution patterns on the trichothecene skeleton affect toxicity. While toxicity varies among different organisms, it is generally agreed that esterification or other modifications of C3 lead to reduced or loss of toxicity. We recently carried out a series of structural studies by high resolution NMR on a series of Type A and B trichothecenes, which are associated with FHB of wheat and barley. Our results show that a water molecule is tightly bound to the B-ring of the toxins, interacting directly with the proton at C3. It is hypothesized that this interaction is important to trichothecene toxicity. Here, we present our recent structural analysis data, complemented with *in vitro* translation initiation inhibition assays of four trichothecenes with different substitution patterns in their functional groups.

P-31

Survey for *Fusarium graminearum* 15-ADON, 3-ADON and NIV chemotypes in winter wheat in Ontario

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The two main chemotypes of *Fusarium graminearum* (FG) found in Canada are 15-acetyldeoxynivalenol (15-ADON) and 3-acetyldeoxynivalenol (3-ADON). The objective of this study was to survey winter wheat in Ontario in 2014 and to identify the frequency of FG chemotypes (15-ADON, 3-ADON and Nivalenol- NIV). Grain samples from six winter wheat cultivars were collected from Ontario Performance Trial. Locations were Ottawa, Palmerston and Ridgetown, while cultivars were AC Morley, Ava, Princeton, Emmit, Wentworth and Pioneer 25R40. One hundred and fifty kernels of each cultivar were surface - sterilized in 0.16% NaOCl (diluted commercial bleach) for three minutes, air dried, and plated on acidified potato dextrose agar. The kernels were incubated for seven days under a 12:12 hr light: dark cycle at room temperature. Single spore cultures of FG were recovered (144 in total) and used for genomic DNA extraction. FG isolates were identified morphologically and using specific molecular markers. 15-ADON, 3-ADON and NIV chemotypes of the fungal strains were identified using *TRI3*- and *TRI-12* based molecular markers. The percentage of FG 15-ADON chemotype was 63.9%, 95.8 % and 97.9 % from Ottawa, Palmerston and Ridgetown, respectively. 2.1% of NIV was detected at both Palmerston and Ridgetown; NIV was not detected in Ottawa. 3-ADON was detected at 36.1% in Ottawa, only 2.1% at Palmerston and was not detected at Ridgetown. We concluded that the frequency of the FG 3-ADON chemotype in winter wheat in Ottawa was much higher in 2014 (36.1%) than recorded previously (2%- 14.6%) from 2004, 2008, 2011 and 2013 in Ontario.

4. Resistance Breeding

P-32

Using GGE biplot data analysis to detect the components of resistance in barley for fusarium head blight through *in vitro* grain assay

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Fusarium head blight (FHB), incited by *Fusarium graminearum*, causes considerable yield and quality losses in barley (*Hordeum vulgare* L.) worldwide. Resistant barley may be used as part of an integrated program for disease management. In the present study, a grain assay using various barley genotypes was conducted to identify the components of resistance including seedling mass weight, germination, coleoptile area and deoxynivalenol (DON) *in vitro* after seed was inoculated with *F. graminearum*. Data were also obtained on these genotypes from the field nursery at Brandon for FHB visual ratings and DON content of the grain. All the data were statistically analyzed and visually displayed using a GGE biplot to identify genotype resistance and the interactions between methods and genotypes. The first two principal components accounted for 62.4 to 82.2% of the total GGE variation for the components of resistance measured. From the bi-plot analyses, DON *in vitro* tended to be negatively correlated with seedling mass weight, germination, coleoptile area, while the latter three components were positively correlated with each other. Most of DON data from field grain samples were positively correlated among FHB nurseries. Field visual rating data may or may not be correlated with the *in vitro* component data from the grain assay. Resistant genotypes were identified based on resistant components using the grain assay. However in most cases, the DON *in vitro* measurement was not correlated with the amount of DON from field grain samples. Further study is needed to improve the efficiency in measuring *in vitro* components for the selection of resistant genotypes.

P-33

Haplotype analysis for fusarium head blight resistance in a collection of Brazilian spring wheat

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Genetic variability is a crucial element in any wheat breeding program. In 2014, 81 wheat lines from various Brazilian breeding institutions were received as part of a Canada-Brazil germplasm exchange. It was anticipated that the material would provide a valuable new source of disease resistance, particularly for fusarium head blight (FHB) and leaf rust. Seventy-nine of the Brazilian lines with spring wheat habit were genotyped using DNA markers (SSR, STS and KASP) at three important FHB quantitative trait loci (QTL). These QTL were previously reported on chromosomes 3BS, 5AS and 6BS respectively, in cultivar Sumai3. In addition, lines were genotyped for KASP and SSR markers at three reduced-height genes (*Rht-B1*, *Rht-D1* and *Rht8*). Visual Ratings Index (VRI) for reactions to FHB were evaluated in the Ottawa inoculated nursery in 2015 and 2016, and deoxynivalenol (DON) content was determined from 2015 harvested nursery samples. The Brazilian collection does not possess the Sumai3 haplotype for FHB resistance QTL on 3BS (*Fhb1*) or 6BS (*Fhb2*). While 63% of the lines amplified the Sumai3 allele at gwm415 on 5AS, none amplified the Sumai3 allele at adjacent SSRs. Nevertheless, VRI ranged from 2.0 to 95.0 in 2015 and 13.3 to 95.0 in 2016. Similarly, DON levels ranged from 1.3 to 35.0 ppm. The Brazilian collection appears to possess FHB resistance not associated with the three major Sumai3 FHB QTL. A significant ($P < 0.05$) negative correlation between plant height and VRI was found but no significant relationship between plant height and DON was observed.

P-35

***In vitro* selection of barley microspores for increased deoxynivalenol tolerance**

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Microspore embryogenesis is routinely used in our breeding program to identify lines with superior tolerance to FHB and deoxynivalenol (DON) accumulation but this traditional breeding approach requires several years of effort. *In vitro* selection is a method that can offer the possibility to rapidly select genotypes with a cellular tolerance to DON. In a first experiment, we investigated four concentrations of DON to determine the best one to be used in view of imposing a strong selection pressure on microspores from 4 barley genotypes (Synasolis, Océanik, Gobernadora and Myriam). At 3×10^{-5} mg/ml of DON, Synasolis exhibited a high level of *in vitro* tolerance to DON by producing more embryos and green plants than the other genotypes tested. These results suggested a potential cellular tolerance in Synasolis and also the possibility to select for this tolerance *in vitro*. Secondly, using these same conditions, we screened an additional thirty barley genotypes. Thirdly, we crossed Synasolis with eight barley cultivars showing good field DON tolerance, but no cellular tolerance in the hope of combining these resistance mechanisms. We performed *in vitro* selection on F1 microspores from these crosses. The F1 microspores were divided into two groups; one served as a control (no DON), while the others were placed on medium containing 3×10^{-5} mg/ml of DON. The objective was to obtain fifty control lines and ten *in vitro*-selected lines to determine if such *in vitro*-selected DH lines exhibit improved field tolerance to DON accumulation.

P-36

Evaluation of genomic selection as a breeding method for developing FHB resistance and reducing DON accumulation in two-row barley

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Fusarium head blight (FHB) caused primarily by *Fusarium graminearum* Schwabe, continues to be the most devastating disease of barley (*Hordeum vulgare* L.) in Canada. Mycotoxins produced by the fungus render grains unsuitable for use in malting and brewing or livestock feed industries. Breeding for FHB resistance and lower deoxynivalenol (DON) content have been long-term goals of Canadian barley breeding programs. Achieving these objectives has been challenging due to the lack of major genetic resistance. While several moderately resistant barley cultivars have been released, stringent industry standards demand ongoing breeding efforts to enhance resistance. Genomic selection is being evaluated as a breeding method to assist in the development of new resistant varieties. Over two growing seasons (2014 & 2015) a large, diverse training set of two-row barley lines were grown in three, irrigated FHB nurseries across Manitoba (Brandon, Carberry and Carman). Lines were scored for visual FHB symptoms (0-5 scale) and quantified for DON content via enzyme-linked immunosorbent assay. The training set will be genotyped using a new 50K SNP marker array (Illumina iSelect HTS Custom Genotyping BeadChips). Genome-wide markers will be used to predict genomic estimated breeding values (GEBV) for breeding populations. These populations have been grown in FHB nurseries in 2016 and will be used to validate accuracy of GEBV's. If successful, genomic selection could be implemented as a general protocol at Agriculture and Agri-Food Canada's Brandon Research and Development Centre to help reduce reliance on labour-intensive FHB nurseries.

5. Epidemiology & Disease Management

P-37

Quantitative comparisons of species within fusarium head blight disease complex affecting oat

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In recent years, fusarium head blight (FHB) has emerged to be one of the most important diseases of oats, especially in the Canadian Prairies. Several *Fusarium* species can infect oats and produce different mycotoxins in contaminated grains which can cause immunosuppression and various health issues when consumed. This has raised concerns among industries and producers since oats have become desirable for human consumption due their high nutritional value. Of special interest among *Fusarium* mycotoxins are the trichothecenes (e.g., T-2 and HT-2 toxins produced by *F. sporotrichioides*), deoxynivalenol (DON, mainly produced by *F. graminearum* and *F. culmorum*) as well as beauvericin and enniatin (mainly produced by *F. poae*). In this study, we surveyed *Fusarium* species infecting oats in Manitoba from 2014 to 2016. Oat samples were collected from commercial oat fields in Manitoba. Species identification was performed based on morphological characteristics and species-specific PCR. *Fusarium* biomass in contaminated grains was assessed by real-time qPCR using primer sets specific to *F. poae*, *F. graminearum* and *F. sporotrichioides*. In addition, we inoculated two oat varieties (Legget and Dancer) with *F. poae*, *F. graminearum* and *F. sporotrichioides* either individually or in combinations. The preliminary results show that *F. poae* is the most common *Fusarium* species found in field samples. In addition, the presence of synergistic effects when oat kernels are infected by more than one *Fusarium* species indicates that *Fusarium* species infecting oat are more diverse than those infecting wheat and *Fusarium* mycotoxins other than DON should be considered.

P-38

Using hyperspectral imagery to track FHB infection in resistant and susceptible wheat varieties

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Fusarium head blight (FHB) is a fungal disease of cereal crops that affects kernel development, grain yield and grain quality. *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein. Petch) is the most prevalent causal FHB pathogen in North America. Infection can lead to the production of the mycotoxins 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) depending on the *F. graminearum* chemotype. Traditionally, Type I and Type II resistance to FHB damage is assessed using subjective visual inspection. In this study we have examined the potential of hyperspectral digital imaging (UHD-185 Firefly, Cubert GmbH., Germany) as a quantitative technique to assess fungal spread. We used point inoculation of spikes at 50% flowering with 3-ADON and 15-ADON chemotypes in a mixed inoculum of 100,000 spores per microliter to track infection in common bread wheat cultivars Roblin, a highly susceptible cultivar, Waskada, a moderately resistant cultivar, and Sumai3, a highly resistant cultivar. Infection was documented over a 14-day period in three biological replicates as well as in mock infected plants. Spectral changes around 550 nm and 700 nm were most sensitive in detecting kernel damage and infection spread throughout the spike. This study shows that differences in infection damage can be accurately tracked using hyperspectral imaging and suggests that hyperspectral technology may be a high throughput, highly accurate, low cost approach to support FHB breeding and research.

P-39

Post-registration assessment of fusarium head blight resistance levels in spring wheat varieties

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Fusarium head blight (FHB) is a major disease of wheat in Manitoba and can impact producer's profitability through reduced grain yield and quality. Varietal resistance is one method used to mitigate losses due to FHB. Testing to determine a variety's FHB resistance is conducted during the three years the variety is in the Variety Registration Trials. However, disease data generated for variety registration provide limited comparisons with other registered varieties over limited locations. This study began in 2009 to evaluate the effect of FHB on spring wheat varieties with varying levels of FHB resistance under natural conditions over a wide geographic area in Manitoba. Harvested samples of varieties from various classes were collected from Manitoba Crop Variety Evaluation Team (MCVET) at various locations from 2009 to 2015. Samples were analyzed for fusarium damaged kernels (FDK) and deoxynivalenol (DON). Mean FDK and DON were determined using mixed model analysis. Analyzing FDK and DON data using this method allows direct comparisons of varieties which may not have been tested together and adjusts for factors such as year, location and interactions. Results will provide post-registration information on reaction of current and new varieties to FHB, with the aim to improve the decision-making capability of producers and agronomists.

P-40

Development of a rapid and efficient laboratory greenhouse artificial inoculation system to measure resistance of barley, wheat and oats to *Gibberella zeae* (Schwein.) Petch

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Fusarium head blight is one of the most economically important agricultural diseases in the world. Extensive research was conducted and reported on the epidemiology and etiology of the primary causal agent of FHB, *Gibberella zeae* (anamorph: *Fusarium graminearum*), and protocols were developed to study this pathogen in laboratory, greenhouse and field settings. We tested different inoculation and disease assessment methods to identify the most rapid, repeatable and efficient system for laboratory artificial inoculation. Four *G. zeae* isolates were initiated on potato dextrose agar (PDA). Colonized agar plugs were used to inoculate V8 juice, tomato juice, mung bean extract or potato dextrose broth at different concentrations. Conidia harvested from each media source were used in a leaf clipping analysis to determine virulence. Initial virulence assays focused on a small number of barley cultivars, but additional registered barley, wheat and oat cultivars will be tested in future with the results from virulence assays being compared with published results from regional field tests. The clip assay involves growing plants in small pots at high density for 7-10 days then trimming the leaves to standard heights and spraying with suspended conidia. Disease severity was determined by measuring the length of necrotic lesions at different time points and DNA quantification will be used to determine the amount of *G. zeae* DNA in each infected leaf. The primary goal is to produce a comprehensive method for disease assays where biocontainment is essential and to supplement field research on disease management strategies or resistant cultivar development.

P-41

Strategies for fusarium head blight management in spring wheat

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Fusarium head blight (FHB), is considered the most serious disease affecting wheat, causing significant yield losses worldwide. Yield losses are caused by fungal infection, but grain quality is significantly reduced due to Fusarium-damaged kernels (FDK) and contamination of grain with the trichothecene mycotoxin deoxynivalenol (DON). There is currently no strong single control strategy to manage either FHB or mycotoxin contamination in spring wheat. There has been evidence that a combination of practices, such as crop rotation, tillage, variety selection and fungicide use and timing, reduce FDK and DON levels in an additive manner. The objective of this research was to evaluate the impact of various seeding rates, variety selection, fungicide timing and need for fungicide application on FHB management, as well as yield response due to leaf control. A total of 63 combinations of management practices in spring wheat were evaluated in two locations. Data collection included plant stand count, wheat growth stages, leaf health rating, disease incidence and severity, yield, protein content, FDK and DON content.

P-42

Pathogenicity of *Fusarium graminearum* isolates collected from wheat fields in Manitoba

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Fusarium head blight (FHB) is caused by various *Fusarium* spp. of which the most important is *Fusarium graminearum*. FHB causes significant losses in grain yield and quality, and affects food and feed safety by producing mycotoxins such as deoxynivalenol (DON) in the grain. Monitoring changes in the pathogen population is critical to ensure breeders are using the right genes to breed for resistance. The objective of this study was to evaluate the pathogenicity of four *F. graminearum* isolates collected from wheat fields in Manitoba in 2015 and four isolates currently being used by breeders for disease screening. Growth characteristics were evaluated in culture and the ability to induce disease symptoms on a group of wheat genotypes with known FHB reaction was assessed in FHB nurseries in Brandon, Carman and Morden. The results showed changes in the aggressiveness of *F. graminearum* isolates among wheat genotypes and locations.

P-43

A role for dihydroquercetin in the control of fusarium head blight in wheat

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Fusarium head blight (FHB) is a devastating disease affecting both yield and quality of wheat grain in Canada and around the world. While FHB-resistant cultivars are available, the mechanism of resistance remains poorly understood and the transfer of strong resistance into elite varieties remains a challenge. Plant secondary metabolites are known for their antimicrobial activities and the flavonoid pathway intermediate dihydroquercetin (DHQ) has previously been reported to have a significant impact on the growth of a number of *Fusarium* species including the primary cause of FHB in Canadian wheat, *Fusarium graminearum*. The goal of this study is to evaluate DHQ as a target of novel techniques such as gene editing to increase FHB resistance. The growth of *F. graminearum* on solid media was significantly reduced when DHQ was present and point inoculation of the pathogen into wheat spikelets with the metabolite led to a significant reduction in pathogen symptoms compared to the control. Two enzymes in the flavonoid pathway, dihydroflavanol reductase and dihydroflavanol 4-reductase, were targeted using virus induced gene silencing to evaluate the potential to increase levels of the metabolite in wheat spikes using a gene knockout approach. A significant reduction in transcript abundance was achieved for both genes and efforts are currently underway to quantify changes in metabolite abundance using LCMS.

P-44

Cropping factors influencing *Fusarium* species and mycotoxins in grain maize –a Swiss survey

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Maize is frequently infected by a complex of *Fusarium* species causing root, ear and stem rot, resulting in yield losses, reduced seed quality and contamination with mycotoxins, causing severe animal health problems. To assess the risk of *Fusarium* and mycotoxin contamination in Swiss grain maize, a survey was conducted (2008-2010) to analyse the *Fusarium* incidence, mycotoxin content and the influence of different cropping measures. *Fusarium graminearum* (FG), *F. verticillioides*, *F. proliferatum* and *F. subglutinans* were the most frequent species and their prevalence varied substantially from year to year. Deoxynivalenol (DON) was the dominant mycotoxin and 51% of all samples exceeded the European DON guidance value for pig feed. Levels of zearalenone and fumonisins were considerably lower.

Analyses of all cropping measures showed that harvest date had the greatest impact: late harvest (end of October or later) led to up to six fold higher DON contents than earlier harvest (mid/end of September). Hybrids of mid-late maturity showed higher DON contents than hybrids of early/mid-early maturing classes. Furthermore, reduced tillage significantly increased infection and mycotoxin contamination compared with samples from ploughed fields, irrespective of the previous crop. Pre-previous crop “cereals or maize” significantly increased the risk of DON in samples from ploughed fields. Since effects of hybrids and pre-crops might have been masked by the highly variable cropping conditions, we also examined maize samples from Agroscope variety trials (2011-2013). These analyses demonstrated that previous crop maize increased FG infections and DON contents. More importantly, we were able to reveal differences in hybrids with DON mean values ranging from 0.6 to 1.9 ppm, irrespective of the maturity class.

P-45

Evaluation of fusarium head blight mitigation through the use of the biological control agent, *Clonostachys rosea*

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The use of biological control agents has gained popularity among consumers as a perceived safer option to prevent disease as opposed to chemical controls. Research has shown that *Clonostachys rosea*, a soil born fungus, has the ability to decrease the levels of fusarium head blight (FHB) and deoxynivalenol (DON) in wheat. This experiment investigated the rate and time of application needed for this biocontrol agent to show beneficial results against FHB, caused by *Fusarium graminearum*, when sprayed topically onto wheat spikes and leaves. Replicated trials were conducted in the 2015 and 2016 growing seasons in Winnipeg, Manitoba. The efficacy of the biological control was compared on different spring wheat genotypes ranging in resistance to FHB. The efficacy was also compared to the chemical control agent, metconazole. Preliminary results concluded that earlier application at the flag leaf stage decreased the presence of the disease compared to applications at the heading or anthesis growth stages. However the efficacy of the product, at any rate or time of application, was lower than the chemical control agent.

P-46

The interaction effect of *F. graminearum* chemotypes and spring wheat genotypes on type II resistance

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Fusarium graminearum, the causal agent of fusarium head blight on wheat, produces deoxynivalenol (DON) which is toxic for humans and animals. The DON producing strains are divided into 3-acetyl (3A) DON and 15-acetyl (15A) DON chemotypes. In Canada 3-ADON is more dominant and aggressive compared with 15-ADON. The objective of this study is to examine the interaction of *F. graminearum* chemotypes collected from across Canada with genotypes of spring wheat having different levels of resistance against FHB. Eight spring wheat genotypes with different levels of resistance against FHB were point-inoculated at anthesis with 10 isolates of *F. graminearum*, of which 5 isolates were 3-ADON- and 5 were 15-ADON-producing isolates. Numbers of infected spikelets were recorded at 7, 14 and 21 days after inoculation, from which the Area Under the Disease Progress Curve (AUDPC) was calculated. While the effect of wheat genotype seemed to be the major source of variation, the effect of *F. graminearum* isolates and the interaction effects were not significant. Results indicate that evaluation of breeding materials for Type II resistance in wheat may be independent of isolate or chemotype.

P-47

Towards adaptation of a detached head assay for measuring resistance to initial infection (Type 1 resistance) by *Fusarium graminearum* in wheat

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Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum* and related species, results in economic loss and grain quality reduction. Disease resistance is typically reported as FHB index, which is a combined measure of incidence (percentage of diseased heads) and severity (percentage of diseased spikelets within diseased heads). FHB index, evaluated through spray or grain spawn inoculation, provides a measure of resistance that is sometimes correlated with resistance to initial infection (Type 1 resistance). However, it does not give an accurate measurement of resistance to initial infection because the value of FHB index is influenced by other forms of resistances, such as disease spread (Type 2 resistance). Disease incidence can be equated to some extent with Type 1 resistance, but a lack of uniform exposure of heads within a plant or a plot affects the reliability and reproducibility of results. Greenhouse disease evaluations offer a higher level of control, but do not eliminate the variability inherent to spray inoculation methods. We are modifying a detached head assay, which we are combining with histological assessments in an effort to establish an improved protocol to assess the mechanisms of Type 1 resistance. This method will be employed to assess the role of hormone signalling in the wheat response to *Fusarium graminearum*.

P-48

Phenotyping of a diverse panel of Canadian winter wheat for fusarium head blight resistance

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Fusarium head blight (FHB), caused by *Fusarium graminearum* (FG), is one of the most detrimental diseases of wheat, worldwide. Since late 1920's there have been at least eight major FHB epidemics in Ontario. The objective of this study was to phenotype a Canadian Winter Wheat Diversity Panel (CWWDP) for FHB response. In 2015, 200 genotypes were artificially inoculated using conidial suspension point inoculations in a growth room (GR) and conidial spray inoculations at the University of Guelph Elora Research Station (ERS). Number of infected spikelets (NIS) in GR were recorded 7, 10, and 14 days after inoculation (DAI), from which the area under disease progress curve (AUDPC) was calculated. Disease incidence and severity, and fusarium damaged kernels (FDK) after harvest were recorded in the ERS, based on which weighted index values were estimated for each experimental unit. No correlation between the GR and ERS data was observed. Independent analysis of GR data showed that NIS at 7DAI does not correlate strongly with other parameters, thus emphasizing the importance of observing disease progress through 14DAI or more. ERS data revealed no correlation of FDK with disease incidence or severity ratings. Moreover, a majority of the genotypes appeared susceptible under high disease pressure conditions. Indices derived from weighted contribution of recorded variables may be ideal for genotype selection decisions. SNP genotyping of the panel for genome-wide association studies is under way.

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