5th Canadian Workshop on Fusarium Head Blight/ Colloque Canadien Sur La Fusariose

Delta Winnipeg
Winnipeg, Manitoba
Nov. 27th to 30th, 2007

National Committee:
Andy Tekauz (Chair)  Richard Martin
Brent McCallum (Registrar/Treasurer)  Shea Miller
Lily Tamburic-Ilinic (Poster Session Chair)  Jennifer Mitchell-Fetch
Randy Clear  Penny Pearse
André Comeau  Brian Rossnagel
Dilantha Fernando  Marc Savard
Jeannie Gilbert  Daryl Somers
David Kaminski  Kelly Turkington
Harvey Voldeng

Proceedings Compiled by Randy Clear, Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main St., Winnipeg, Manitoba

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Greetings

On behalf of the National and Local Organizing Committees (NOC, LOC), welcome to the 5th Canadian Workshop on Fusarium Head Blight (5th CWFHB), Winnipeg, Manitoba, November 27-30, 2007. As has been the case since the inaugural workshop in 1999, we will have some 200 delegates in attendance, attesting to the continued interest and research effort being targeted against this disease of regional, national and indeed global importance.

The NOC has worked diligently over the past year to assemble a stimulating and informative scientific and technical program, and some 30 invited talks are to be presented in seven topic sessions. The Genomics and Genetics session, in particular, is most comprehensive on this occasion. In addition, 60 poster abstracts covering all topic areas have been submitted by you, the delegates, and the full posters to be shown at the workshop will expand greatly on the total information package available. The NOC has endeavoured to address many of the current concerns and research advances relating to FHB, thus making the program appealing to as broad an audience as possible, i.e. researchers, industry, producers, consumers, and others. At the same time, the LOC has arranged for a social program tailored to foster maximum dialogue and discussion by allowing participants to remain together during most non-scientific segments of the day.

I also want to take this opportunity on behalf of all of you, to thank our generous sponsors who by partnering with us have demonstrated their own commitment to mitigating the effects of this devastating disease. Their support facilitates continued cooperation and collaboration among participants, has enabled us to enlist a roster of key invited speakers, and has kept registration fees reasonable making the 5th CWFHB accessible to as many delegates as possible.

I trust you will enjoy all aspects of the workshop and depart stimulated to continue your valuable contributions to defeat FHB. If you have any comments or suggestions, please feel free to contact any of the Organizing Committee members during or following the workshop. Sincere thanks to all of you for your continued support of the CWFHB.

Andy Tekauz
Chair, Organizing Committees 5th FHB
The Brewing and Malting Barley Research Institute (BMBRI) is an industry organization representing major malting and brewing companies. It was incorporated in Canada in 1948. The nine current members are: **Canada**: International Malting Company Canada, Prairie Malt, Rahr Malting Canada, Labatt Brewing Company, Molson Canada, Moosehead Breweries, Sleeman Breweries; **USA**: Anheuser-Busch, Sierra Nevada Brewing Company.

The main focus of the organization is to ensure that malting barley varieties which will meet the needs of member companies are developed, evaluated and registered for commercial production in Canada. This is accomplished by participating in variety evaluation trials from very early stage through plant scale trials, financially supporting research projects which will provide knowledge and technologies to the breeding programs (recent grants are listed on the BMBRI web site: [www.bmbri.ca](http://www.bmbri.ca)), communicating industry needs to breeders and researchers, and participating in the variety registration system in western Canada. Through the BMBRI, members are represented at a number of other tables throughout the broader system, including the Barley Development Council, the Barley Advisory Committee of the Western Grains Research Foundation, and the Western Grains Standards Committee and Barley Subcommittee. An additional area of activity is communicating with barley producers to emphasize the importance of the quality of their crops to the malting and brewing industry.
Statement:
"CANTERRA SEEDS is Always Growing ... As seed experts, as innovative agronomists and as a business dedicated to the success of producers, farm families and rural communities. A Canadian-owned company with more than 170 shareholders, our roots have spread globally with research, development and valuable relationships with plant breeders from around the world. We are committed to service, quality certified seed and superior genetics. Firmly planted in Canada but with our eye on the globe, we are always growing.

Mission Statement:
To use our extensive knowledge and experience to act as SEED EXPERTS when acquiring, producing, marketing and selling seed and related technologies to our customers.
To be a leader in identifying opportunities and source QUALITY products and varieties that meet "end user" needs.
To work with our shareholders, retailers, customers and partners to identify OPPORTUNITIES that allow us all to succeed in the agriculture industry."

Manitoba Pork Council is the membership association of all hog farmers in the province. Its mission is to foster the sustainability and prosperity of the pork industry for the good of hog farmers and all Manitobans.

Manitoba’s hog industry is a success story we can all be proud of. Over the last two decades, Manitoba hog farmers have built a strong industry that has put our province on the map as a centre of excellence for pork production. Manitoba hog farmers put a top-quality product on the table for consumers at home and around the world. The end result has been job creation, growing communities and a significant economic contribution to Manitoba.

Not only has hog farming created more than 15,000 jobs for Manitobans, hog famers also add about $1 billion to the provincial economy each year. While this has made the hog industry a key player in our provincial economy, it is not what has been accomplished to date – it is what lies ahead.
Manitoba’s 1200 hog farmers share a vision for the future that will see us move beyond our current markets and into exciting new opportunities, enabling us to continue building an industry that is sustainable for future generations.

Manitoba Pork Council’s long-term vision for success is based on three main areas of focus: 1. Sustainable Industry; 2. Economic Value; and 3. Research & Technology.

Syngenta Crop Protection Canada, Inc. is a leading global agribusiness company committed to sustainable agriculture through innovative research and technology. Syngenta Canada provides an extensive range of products and services that span the country’s major crops including wheat, barley, canola, corn, potatoes, pulse crops, and soybeans and ranks third in the high-value commercial seeds market. Syngenta crop Protection, Seed Care and Syngenta Seeds divisions collaborate to provide a complete offering of integrated crop solutions, from expert agronomic advice and best management practices to cutting edge technology and scientific innovation designed to help Canadian growers produce robust yields and high quality crops.
Our Mission and Vision

At BASF, our vision is to be the world’s leading agricultural innovator, optimizing crop production, improving nutrition and enhancing quality of life.

Our mission is to enhance value by delivering products and services that satisfy customers’ needs and contribute to the sustainability of agriculture. Through customer focused partnering, marketing and sales expertise and broad R&D and manufacturing experience, BASF will work hard to earn our customers’ business.

Mission Statement:
To provide innovative, sustainable and profitable crop protection solutions to our customers in Canada through our expertise, technology and products.

The Canadian Wheat Board (CWB) is a farmer-controlled marketer of the more than 20 million tonnes of wheat and barley grown in Western Canada. With annual sales revenues of $4 to $6 billion and customers in over 70 countries, the CWB is the world’s largest single marketer of wheat and barley. With a marketing strategy that concentrates on high quality products and with a clear mandate to help maximize farmer income, the CWB is very supportive of finding solutions to Fusarium Head Blight (FHB). Through CWB direct funding of FHB research projects and through the Western Research Foundation checkoff deduction from CWB payments, Western Canadian farmers provide considerable financial support to breeding research for FHB resistance.
The Ontario Wheat Producers’ Marketing Board represents 17,000 Ontario wheat producers by providing strategic leadership initiatives that promote and improve Ontario wheat. Over 2 million tonnes of wheat are produced in Ontario - Canada’s most diverse region of wheat production. Ontario produces four commercial classes of wheat including soft red, soft white and hard red winter and hard red spring wheat along with two classes in limited production – hard white spring and durum.

The OWPMB is a supporter and partner in many research projects in the province as it is our mandate to add value to all wheat producers. One area of research that is a high priority for our Board and producers is fusarium control. Great strides have been made by the Ontario wheat industry and government not only in variety development but also in management techniques and crop protection programs to address this disease concern for Ontario producers. The OWPMB will continue to support this research within the Ontario wheat industry to ensure even greater strides are made in future research to reduce this disease’s economic impact.

The Manitoba Rural Adaptation Council Inc. (MRAC) is a private not-for-profit corporation that administers federal funds for innovative agricultural projects and acts as a catalyst to stimulate industry and government activity where gaps are identified.

Since its incorporation in 1997, MRAC has funded over 400 projects, contributing over $21 million towards adaptation and innovation. By working with numerous industry partners, including producers, producer organizations, private and public corporations, educational institutions, and government, MRAC has leveraged the investment of over $68 million in agricultural advancement in this province.

MRAC has administered the Canadian Adaptation and Rural Development (CARD) program and currently administers the Manitoba share ($10.3 million) of Agriculture and Agri-Food Canada’s $243 million Advancing Canadian Agriculture and Agri-Food (ACAAF) program. MRAC also administered the Agricultural Environmental Stewardship (AESI) Program and the Canada Agri-Infrastructure Program (CAIP) research funding in Manitoba. In August 2006 MRAC was chosen to deliver the Biofuels Opportunities for Producers Initiative (BOPI) in Manitoba.
Silver Sponsors

Our Purpose
Is to be the global leader in nourishing people

Our Mission
Is to create distinctive value

Our Approach
Is to be trustworthy, creative and enterprising

Our Performance Measures
Are engaged employees, satisfied customers, enriched communities, and profitable growth

A leader in delivering excellence and innovation in grain quality and quantity assurance, research and producer protection.

Dow AgroSciences Canada is an organization that is committed to enhancing the quality of life by developing innovative technology for, efficient production of an abundant, nutritious food supply and the use of renewable agriculture resources for industrial applications.

Dow AgroSciences Canada Inc. is a research based, agricultural sciences company with diverse product portfolio including weed, insect and disease management for agricultural/horticultural crops, forestry and industrial vegetation management. The company has developed a plant genetics and biotechnology platform in canola and corn. This investment is focused on agronomic production traits and value-added quality traits. Dow AgroSciences has capabilities across western and eastern Canada including a plant breeding and cell biology group based in Saskatoon, Saskatchewan and a research farm in St Mary’s Ontario.

Its operating style is based on strategic alliances and industry partnerships. The company has developed several significant research and commercial development alliances in Canada including SemBioSys
Genetics Inc. of Calgary, Performance Plants of Kingston Ontario, Natunola Health Inc. of Ottawa, the National Research Council's Plant Biotechnology Institute in Saskatoon Saskatchewan and with Agriculture and Agri-Food Canada across Canada. Dow AgroSciences Canada Inc. is an affiliate of Dow AgroSciences LLC, a $3-billion global company based in Indianapolis, Indiana. Dow AgroSciences is a wholly owned subsidiary of The Dow Chemical Company.

Vision
The Prairie region will, as a result of the efforts of Genome Prairie and its collaborators, be recognized as a leading center in genomics research and its applications to agriculture (crops and animals) and to human health.

Standard Corporate Profile
Genome Prairie is the leading organization for support and management of large-scale genomics and proteomics research projects in Manitoba and Saskatchewan. With its partners, Genome Prairie has supported more than $120 million of research activity in plant, animal and human genomics, bioinformatics, instrumentation development and bioethics since 2000.

Genome Prairie works collaboratively with all levels of government, universities and industry as well as Genome Canada, a not-for-profit organization implementing a national strategy in genomics and proteomics research to benefit all Canadians.

With offices in Winnipeg and Saskatoon, Genome Prairie continues to work with researchers in both provinces to facilitate genomics and proteomics projects in areas such as agriculture, environment, human and animal health. www.genomeprairie.ca

Main Roles
Genome Prairie has three key roles involving genomics and proteomics research:

1) Manage research projects and facilitate regional participation and co-funding for Genome Canada investments in the Prairie Region.
2) Provide leadership and support for collaborative efforts for genomic research and knowledge transfer for the provinces of Manitoba and Saskatchewan.
3) Provide public education and awareness of ethical, economic, environmental, legal and societal (GE³LS) issues related to genomics and proteomics research.
As “Canada’s Seed Partner”, SeCan actively seeks partnerships that promote profitability in Canadian agriculture. SeCan is the largest supplier of certified seed to Canadian farmers with more than 1,000 members from coast to coast engaged in seed production, processing and marketing. Since its inception in 1976, SeCan has been a major supporter of plant breeding in Canada, returning more than $51 million in royalties and research funding. SeCan represents more than 370 crop varieties developed by public and private sector breeding programs.
Our product is...

**Pedigreed Seed**

Our business is...

**Value Creation**

FarmPure Seeds is a farmer owned globally connected seed distribution and commercialization channel committed to sustainable agriculture. We currently represent 37 public and private breeding institutions across 12 countries.

FarmPure Seeds extensive retail distribution network throughout Canada and the Northern United States is instrumental in its ability to provide broad geographic commercialization of varieties with leading agronomics into traditional commodity markets.

With end-use markets becoming more discerning and fragmented, FarmPure Seeds also specializes in commercializing varieties with unique end-use attributes through identity preservation value chains that provide greater value to all stakeholders.

FarmPure Seeds has one of Canada’s most extensive lines of pedigreed seed offerings in oilseeds, cereals, pulses, grasses and legumes.

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**FPCCQ - MISSION**

Défendre et développer les intérêts économiques et sociaux des membres.

À cet égard, la Fédération exerce les rôles clés suivants :

1. Administrer le plan conjoint des producteurs de cultures commerciales.
2. Influencer le développement des enjeux économiques et technologiques du secteur d’activités afin de favoriser la prospérité et la viabilité des entreprises de grandes cultures et du secteur dans son ensemble.
3. Agir comme leader qui mobilise les partenaires et les gouvernements autour de projets rassembleurs pour le secteur.
4. Développer les habilités des producteurs en matière de production et de commercialisation.

FPCCQ (Fédération des producteurs de cultures commerciales du Québec) protects and develops the economic and social interests of the 11,000 Québec grain producers. In this goal, FPCCQ plays the following key roles:

1. To manage the joint plan of the Québec Cash Crops Growers.
2. To influence the development of economic and technologic issues at stake and help for the prosperity and vitality of members’ farms and the industry as a whole.
3. To act as a leader that mobilizes partners and governments with unifying projects.
4. To develop producers’ ability in production and marketing.

RAHR MALTING CANADA LTD. WILL PRODUCE AND OR DISTRIBUTE PREFERRED MALT AND BARLEY PRODUCTS FOR THE WORLD’S BEVERAGE AND FOOD MANUFACTURERS BY ANTICIPATING AND RESPONDING TO THEIR CHANGING NEEDS IN A WAY THAT DELIVERS TOTAL CUSTOMER SATISFACTION.
Recommendations from the 3rd CWFHB
Dec. 9th to 12th, 2003

Dear Industry and Government Sponsors and Workshop Participants:

At the conclusion of the 2003 meeting, we held discussions on what is needed to forward the struggle against this devastating disease. Based on these deliberations, participants attending the 3rd CWFHB identified the following as priority needs during the next two years:

**Industry/Regulatory**
1. In wheat, to re-examine the requirement for kernel visual distinguishability (KVD) among classes, as less stringent, and alternative identification methods would allow for access to, and more rapid development of improved wheat varieties, including the production of ‘feed’ quality grain for the livestock, ethanol and other value-added industries.
2. For the Canadian Seed Growers Association (CSGA) to consider adding a test for the level of *Fusarium graminearum* and other *Fusarium* spp. as a component of seed certification for the information of the buyer.
3. For representatives of government regulatory agencies to attend future workshops to clarify regulations and interact constructively with other relevant parties to implement such changes to regulatory guidelines as are deemed desirable.
4. That the Canadian Food Inspection Agency’s (CFIA) definition of plants with novel traits as ‘PNT’s be abolished, and that Canada use the same definition for genetically modified organisms (GMOs) used by other countries, i.e. as plants produced by a rDNA transfer, rather than those plants produced by any process, including conventional breeding, but having different or unusual traits.
5. To develop methodologies to mitigate the effects of DON-contaminated byproducts from ethanol production, and the milling, malting and brewing processes, and to identify strategies to enhance utilization of FHB-contaminated grains by the livestock sector.
6. To investigate novel uses for FHB-contaminated grain as safe and useful, value added products.

**Mycotoxins**

7. To significantly expand laboratory facilities and resources available for testing for deoxynivalenol (DON), particularly to expedite progress in developing resistant varieties in breeding programs and to assist in research related to food safety and quality.
8. To develop and validate alternative, rapid methods for detection of DON in whole grain or grain flour (e.g. near infra-red spectrometry - NIR) and for Fusarium-damaged kernels (FDK) (e.g., image analysis), recognizing that different standards are needed for quality control (i.e., high accuracy) vs selection and
screening.

9. For economic tests to screen for mycotoxins other than DON (e.g. nivalenol, DAS, T-2 and HT-2) which can be produced by *Fusarium* spp. other than *Fusarium graminearum*, which contribute to FHB in crops such as barley and oat.

10. For CFIA and Health Canada to expand their surveillance for DON and other mycotoxins in grain, foodstuffs, and other sectors of the market place.

11. To investigate sampling protocols for DON and other mycotoxins, as sampling has been identified as the greatest single source of error in determining accurate, meaningful, and reproducible mycotoxin levels.

12. To standardize analytical methods for detection of mycotoxins among laboratories by use of reference materials to obtain consistent, reliable, and comparable results.

13. To develop a better understanding of the implications of mycotoxin contamination for human health, especially for high risk groups, i.e., infants and children.

**Conventional/Molecular Breeding**

14. To continue investigating FHB in oats, assess its impact on the crop, develop mitigation strategies, and identify sources of resistance for use in breeding programs.

15. To support continued refinement and development of improved phenotyping, i.e., phenomics, to validate the identification of resistant sources, confirm the basis of genetic resistance, and effectively screen breeders’ lines, as well as enable the best use of genomics, proteomics, metabolomics, etc.

16. To fully investigate the pathogenic diversity in the principal causal agent, *Fusarium graminearum*, and evaluate for the occurrence of specialization to different crops and different environments (warm vs cool), both to explain its pervasiveness in existing epidemic areas, and the likelihood of *F. graminearum* moving to new regions, or developing resistance to fungicides.

17. For additional research, in particular histological and histochemical studies, on the mechanisms of resistance, to better understand disease progress and identify weak points where attack on the pathogen can be targeted, or host resistance enhanced.

18. Continue other approaches such as molecular breeding and transformation, re. development of FHB-resistant varieties, as alternative routes to achieve the goal of minimizing the impacts of FHB.

19. For a continued research effort in development of *Fusarium*-resistant corn inbreds, that can be crossed to develop *Fusarium*-resistant hybrids.

**Epidemiology/Disease Management**

20. To clarify the role of corn, both as an additional major host of FHB (pink ear rot and stalk rot) and as a source of abundant and persistent *Fusarium* inoculum for the infection of small grain crops.

21. To study the role of crop residues as sources of primary inoculum, particularly
residues of non-host crops (e.g. canola), to assist in the understanding of disease development, and in formulating effective integrated control strategies.

22. To clarify the impact of management practices (e.g., application of herbicides), and better understand the role of crop residues in pathogen survival for overwintering and increasing inoculum potential the following spring.

23. To identify or develop simple, standardized methods to assess and measure *Fusarium* spore concentrations in the atmosphere and to assist in determining the environmental factors responsible for inoculum production and release.

24. To refine and validate current FHB disease forecasting models for effective and economic fungicide application, and to provide for their timely and universal accessibility.

25. To develop more effective fungicides for management of FHB, and to provide Canadian producers with a registered product(s) for use in barley and oat.

26. For producers, agrologists, and industry to be better informed on the various management strategies available to combat FHB. This includes awareness of the various agronomic practices used (e.g., impact of planting seed infected with *Fusarium*, variety selection, crop rotations), as well as the environmental factors that affect disease (e.g., crop staging relative to spore release and the use of disease forecasting models).

**Common Issues**

27. To develop a committed long-term approach to resolve the FHB problem, one that is not de-railed by complacency arising from one or more years of low FHB.

28. For CFIA and Health Canada to re-examine Canada’s FDK and DON standards and bring these into compliance with the recent or imminent changes to such standards both by our individual customers and international conventions, and to communicate with and assist industry in achieving this.

29. To slow or prevent movement of FHB to new areas (e.g., Alberta) and as such to protect our ability to source unaffected high quality grain over a wide region, maintaining Canada’s competitive advantage.
Destinataires : À l’intention des membres de l’industrie céréalière, des partenaires du gouvernement et des participants aux ateliers.

Mesdames, Messieurs,
À la suite de la réunion, on a entamé une discussion approfondie sur les éléments essentiels permettant de lutter davantage contre cette maladie dévastatrice. À la suite des discussions, les participants au troisième colloque sur la fusariose ont dressé la liste des priorités pour les deux années à venir.

**Industrie céréalière/réglementation**

1. Blé : On doit réexaminer l’exigence liée à la distinction visuelle des grains (DVG) relativement au classement du blé, au fur et à mesure que d’autres types d’épreuves rapides et moins exigeantes permettront de mettre au point plus rapidement des variétés améliorées de blé et d’y avoir accès, y compris les variétés fourragères et celles en vue de la production d’éthanol, ainsi que celles créées pour répondre aux besoins de l’industrie de produits à valeur ajoutée.

2. L’Association canadienne des producteurs de semences (ACPS) doit se pencher sur le processus de certification une épreuve pour déterminer le taux de *Fusarium graminearum* et de d’autres espèces de *Fusarium* afin de fournir l’information à l’acheteur.

3. Les représentants des organismes de réglementation gouvernementaux doivent assister à des ateliers dans le futur afin de clarifier la réglementation et d’interagir de façon constructive avec les autres intéressés, pour faire en sorte que les modifications voulues soient apportées aux lignes directrices réglementaires.

4. Que l’Agence canadienne d'inspection des aliments (ACIA) abolisse ce qu’elle a établi comme définition pour les végétaux à caractères nouveaux (VCN), et que le Canada emploie la même définition que d’autres pays pour les organismes génétiquement modifiés (OGM) (c’est-à-dire, des plantes produites par la technique de transfert de l’ADN et ayant des caractères différents ou inhabituels, plutôt que des plantes produites par tout autre moyen que ce soit, y compris la sélection classique des végétaux).

5. Établir des méthodologies qui permettent d’atténuer les effets de la contamination par la vomitoxine DON des produits dérivés de la production d’éthanol, et des procédés de meunerie, de maltage et de brassage, et cerner des stratégies menant à une meilleure utilisation des grains contaminés par la brûlure de l’épi dans le secteur de l’élevage.

6. Se pencher sur de nouvelles façons d’employer le grain fusarié comme produit utile et sûr, à valeur ajoutée.

**Mycotoxines**

7. Agrandir les installations de laboratoire de façon importante et obtenir plus de ressources permettant d’effectuer l’analyse du désoxyxynivalénéol (DON),
particulièrement pour développer plus rapidement des variétés résistantes dans le cadre de programmes de sélection et pour appuyer la recherche sur la qualité et la salubrité des aliments.

8. Concevoir et valider des méthodes de rechange rapides pour déceler la présence de DON dans le grain ou la farine de grain entier (par exemple, la spectrométrie dans le proche infrarouge - NIRS) et pour détecter les grains endommagés par le *Fusarium* (par exemple, l’analyse d’images), reconnaître que différentes normes sont nécessaires pour le contrôle de la qualité (par exemple, la haute précision) en comparaison des normes pour la sélection et le criblage.

9. Développer des tests peu coûteux pour déceler la présence de mycotoxines autres que le DON (par exemple, nivalénol, *DAS, T-2 et HT-2*) pouvant être produites par des espèces autres que le celle du *Fusarium graminearum*, lesquelles peuvent favoriser la brûlure de l’épi causée par le *Fusarium* dans des cultures d’orge et d’avoine.

10. L’ACIA et Santé Canada doivent élargir leur surveillance du DON et d’autres mycotoxines dans le grain, les produits agroalimentaires et d’autres secteurs du marché.

11. Enquêter sur des protocoles d’échantillonnage du DON et d’autres mycotoxines, car l’échantillonnage a été cerné comme étant la plus grande source d’erreur lorsqu’on doit établir des niveaux précis, significatifs et pouvant être reproduits.

12. Normaliser les méthodes d’analyse utilisées dans les laboratoires pour déceler la présence de mycotoxines à l’aide d’éléments de documentation permettant d’obtenir des résultats uniformes, fiables et comparables.

13. Développer une meilleure compréhension des effets de la contamination par mycotoxines sur la santé humaine, particulièrement pour les groupes à haut risque (par exemple, les bébés et les enfants).

**Sélection classique des végétaux/sélection moléculaire**


15. Appuyer la mise au point continue et l’élaboration de méthodes améliorées du phénotypage, comme la phénomique, permettant de valider l’identification des sources de résistance, de confirmer le fondement de la résistance génétique et de cribler efficacement les lignées sélectionnées, ainsi que la meilleure utilisation de la génomique, de la protéomique, de la métabolomique, etc.

16. Évaluer à fond la diversité pathogénique de l’agent causal principal, *Fusarium graminearum*, et étudier la présence des cas particuliers qui se manifestent dans différentes récoltes et selon différents environnements (froid et chaud) pour expliquer sa présence plus marquée dans des régions d’épidémie et la possibilité que le *F. graminearum* se déplacera vers de nouvelles régions ou qu’il développera une résistance aux fongicides.

17. Poursuivre nos efforts de recherche, et particulièrement les examens d’ordres
histologique et histo chimique relatifs aux mécanismes de résistance, afin de mieux comprendre la progression de la maladie et d’identifier les points faibles pour mieux cibler l’agent pathogène et le détruire, ou encore, améliorer la résistance de l’hôte.

18. Poursuivre d’autres approches telles que la sélection et la transformation moléculaires, c’est-à-dire, développer des variétés résistantes à la fusariose comme solution de rechange, pour minimiser l’impact de la brûlure de l’épi causée par le *Fusarium*.

19. Poursuivre sans relâche les activités de recherche pour développer des lignées de maïs autofécondées résistantes au *Fusarium* qui pourraient être croisées avec d’autres variétés ou espèces pour développer des hybrides résistants au *Fusarium*.

**Épidémiologie/gestion des maladies**

20. Clarifier le rôle du maïs comme hôte principal additionnel de la brûlure de l’épi causée par le *Fusarium* (la pourriture rose de l’épi du maïs et la fusariose de la tige) et comme source abondante et persistante d’inoculum de *Fusarium* pour contaminer les céréales à petit grain.

21. Étudier le rôle des résidus de culture comme source d’inoculum primaire, particulièrement les résidus de cultures qui ne sont pas hôtes (par exemple, le canola), pour nous aider à mieux comprendre le développement de la maladie, et à développer efficacement des stratégies de contrôle intégrées.

22. Clarifier les retombées des pratiques de gestion (par exemple, l’application d’herbicides), et mieux comprendre le rôle des résidus de culture par rapport à la survie hiémale des agents pathogènes et le potentiel accru d’inoculum le printemps suivant.

23. Identifier ou développer des méthodes simples et normalisées pour évaluer et mesurer la concentration de spores de *Fusarium* dans l’atmosphère, et participer à l’étude des facteurs environnementaux favorisant la production et le relâchement d’inoculum.


25. Développer des fongicides plus efficaces pour contrôler la brûlure de l’épi causée par le *Fusarium* et offrir un ou plusieurs produits homologués aux producteurs canadiens pouvant être appliqués aux cultures d’orge et d’avoine.

26. Mieux informer les producteurs, les agronomes et l’industrie céréalière sur les diverses stratégies de gestion à leur portée pour lutter contre le *Fusarium*. Ceci comprend la sensibilisation aux différentes pratiques agricoles employées (par exemple, les conséquences de l’ensemencement de graines atteintes de fusariose, le choix des variétés, la rotation des cultures) ainsi que les facteurs environnementaux qui influent sur la maladie (par exemple, le stade phénologique relativement à la dissémination des spores et l’utilisation des modèles de prévision de la maladie).
Questions communes

27. Développer une approche à long terme faisant preuve d’engagement pour résoudre le problème du Fusarium, soit une approche qui ne serait pas estompée si jamais on enregistrait une faible incidence du Fusarium pour une ou deux années.

28. L’ACIA et Santé Canada doivent examiner de nouveau les normes sur le Fusarium et la présence de DON, et les rendre conformes aux modifications récentes ou éventuelles apportées à ces normes par nos clients et par les organisations internationales, et communiquer avec les représentants de l’industrie et les aider à réaliser ce but.

29. Ralentir la progression du Fusarium ou l’empêcher de se répandre à de nouvelles régions (par exemple, en Alberta) et protéger ainsi notre capacité d’avoir accès à du grain intouché par la maladie sur une grande superficie, afin de maintenir l’avantage concurrentiel que le Canada détient sur le marché.
# AGENDA

## Tuesday, November 27, 2007

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
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<tbody>
<tr>
<td>4:00 pm - 9:00 pm</td>
<td>Registration desk open</td>
</tr>
<tr>
<td>6:00 pm - 9:00 pm</td>
<td>Poster set-up</td>
</tr>
<tr>
<td>7:30 pm - 10:00 pm</td>
<td>Reception (provided)</td>
</tr>
</tbody>
</table>

## Wednesday, November 28, 2007

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 am - 5:30 pm</td>
<td>Registration desk open</td>
</tr>
<tr>
<td>7:00 am - 10:00 am</td>
<td>Poster set-up</td>
</tr>
<tr>
<td>7:00 am - 8:15 am</td>
<td>Continental breakfast buffet (provided)</td>
</tr>
<tr>
<td>8:15 am - 8:30 am</td>
<td>Welcome Address: “The DON of a New Era in Fusarium Head Blight”. Dr. Barry Todd, Deputy Minister (Manitoba Agriculture, Food and Rural Initiatives, Winnipeg MB)</td>
</tr>
</tbody>
</table>

### 8:30-10:00 Plenary Session/ Plénière:

**Chairs: Jeannie Gilbert, Dilantha Fernando**

**8:30** Diversity of *Fusarium* in Canada: unexpected species and their significance for molecular diagnostics of grain pathogens.  
Keith Seifert (AAFC - ECORC, Ottawa ON)

**8:50** Strategies for improving fusarium head blight (FHB) resistance in European wheat.  
Thomas Miedaner (University of Hohenheim, Germany)

**9:10** Funding Fusarium research: the farmer’s perspective.  
Bill Toews (CWB/Producer, Kane MB)

**9:30** FHB and the bioethanol industry: what is needed?  
Anita Brulé-Babel (University of Manitoba, Winnipeg MB)

### 10:00-10:30 Refreshment Break
10:30-12:00  Mycotoxins/Mycotoxines:  
Chair: Marc Savard

10:30  Recombinant antibodies with affinity for deoxynivalenol: a novel tool against fusarium head blight.  
Patrick Doyle (University of Guelph, Guelph ON)

10:50  Challenges for control and certification of mycotoxins in grain shipments: Canadian experiences with ochratoxin A and their possible implications for Fusarium toxins.  
Tom Nowicki (Grain Research Laboratory, Winnipeg MB)

11:10  Where we should be after three Fusarium disasters in 20 years - 1986, 1996, 2006.  
Art Schaafsma (University of Guelph, Ridgetown ON)

11:30  Toxigenicity and pathogenicity of Fusarium poae and Fusarium avenaceum isolates in wheat - results of field, climate chamber, and laboratory studies.  
Susanne Vogelgsang (ART, Zurich, Switzerland)

12:00-1:30  Lunch provided

1:30-3:00  Breeding Session 1:  Resistance Breeding/ Amélioration de la Résistance  
Chairs: Jennifer Mitchell Fetch, Brian Rossnagel, Harvey Voldeng

1:30  Association mapping in barley for FHB resistance.  
François Belzile (Université Laval, Ste-Foy QC)

1:50  The application of marker-assisted selection for breeding FHB resistant wheat in Canada.  
Gavin Humphreys (AAFC-Cereal Res Centre, Winnipeg MB)

2:10  Progress in Fusarium Head Blight Resistance Breeding in Canadian Durum Wheat.  
Curtis Pozniak (University of Saskatchewan, Saskatoon SK)

2:30  Progress in breeding for FHB/DON resistance in malting barley.  
Linnea Skoglund (Busch Ag Resources, Fort Collins CO, USA)

3:00-3:30  Refreshment Break
3:30-4:30  Breeding Session 2: Panel Discussion  
*Chairs: Jennifer Mitchell Fetch, Brian Rossnagel, Harvey Voldeng*

3:30  Prospects for incorporating FHB resistance in grain crops.

**Wheat - Canada**  
Pierre Hucl (University of Saskatchewan, Saskatoon SK)

**Wheat - USA**  
Mohamed Mergoum (North Dakota State University, Fargo ND, USA)

**Barley - Canada**  
Bill Legge (AAFC-Brandon Research Centre, Brandon MB)

**Barley - USA**  
Kevin Smith (University of Minnesota, St. Paul MN, USA)

**Oat**  
Jennifer Mitchell Fetch (AAFC-Cereal Research Centre, Winnipeg MB)  
Weikai Yan (AAFC-ECORC, Ottawa ON)

**Corn/Maize**  
Lana Reid (AAFC-ECORC, Ottawa ON)

4:30 pm - 5:30 pm  **Poster Viewing 1** (authors to be present); cash bar

5:30 pm  **Dinner** (on your own)
Thursday, November 29, 2007

7:00 am - 5:00 pm  Registration desk open
7:00 am - 8:15 am  Continental breakfast buffet (provided)

8:15-10:00  Physiology of Resistance/ Physiologie de la Résistance:
Chair: André Comeau

8:15  Finding resistance to fusarium head blight (FHB) in unexpected places.
Steve Haber (AAFC-Cereal Res Centre, Winnipeg MB)

8:50  An improved strategy for breeding FHB-resistant wheat must include type 1 resistance.
Akos Mesterhazy (Cereal Research Non-profit Co., Szeged, Hungary)

9:25  Metabolomics as a tool to decipher the mechanisms and for high throughput screening of wheat resistance to FHB.
Ajjamada Kushalappa (Macdonald Campus, McGill University, Ste. Anne de Bellevue QC)

10:00-10:30 Refreshment Break

10:30 – 12:00  Genomics and Genetics/ Génomique et Génétique Session 1:
Chair: Daryl Somers

10:30  Rapid identification of genes contributing to FHB resistance in wheat.
Steve Scofield (USDA-ARS, Purdue Univ, West Lafayette IN, USA)

10:50  Regulation of the trichothecene pathway in Fusarium graminearum.
Gopal Subramanian (AAFC-ECORC, Ottawa ON)

11:10  Targeting scab with defence regulatory genes.
Jyoti Shah (Kansas State University, Manhattan KS, USA)

11:30  Proteome and transcriptome analysis of the impact of trichothecenes and Fusarium graminearum in barley.
François Eudes (AAFC-Lethbridge Res Centre, Lethbridge AB)

12:00-1:30 Lunch provided
1:30 – 2:30  Genomics and Genetics/ Génomique et Génétique Session 2:  
Chair: Daryl Somers

1:30  The search for QTL to employ in marker assisted selection for FHB resistance in barley.  
Kevin Smith (University of Minnesota, St. Paul MN, USA)

1:50  New insights into the biochemical nature of fusarium head blight resistance using proteomics.  
Chris Rampitsch (AAFC-Cereal Res Centre, Winnipeg MB)

2:10  GLK1, a novel transcription factor that confers Fusarium graminearum resistance to Arabidopsis: implications to FHB resistance in wheat.  
Jas Singh (AAFC-ECORC, Ottawa ON)

2:30-3:00 Refreshment Break

3:00 – 5:00 Epidemiology and Disease Management/ Epidémiologie et Contrôle:  
Chairs: Kelly Turkington and Richard Martin

3:00  Dry heat disinfection versus seed-borne Fusarium: five years’ practical experience.  
Dave Gehl (AAFC-Research Farm, Indian Head SK)

Char Hollingsworth (University of Minnesota, St Paul MN, USA)

3:50  Integrated strategies for FHB management, a Northern Great Plains perspective.  
Marcia McMullen (North Dakota State University, Fargo ND, USA)

4:15  Risk reduction strategies for FHB and DON contamination in a high moisture environment.  
Richard Martin (AAFC-CLRC, Charlottetown PEI)

4:40  Meteorological-based systems for predicting and managing Fusarium head blight epidemics in the wheat growing area of Argentina.  
Ricardo Moschini (INTA, Castelar, Argentina)

5:00 pm - 6:00 pm  Poster Viewing 2 (authors to be present)

6:30 pm – 10:00 pm  Dinner/Banquet (provided)
Friday, November 30, 2007

7:00 am - 12:00 noon  Registration desk open
7:00 am - 8:30 am  Hearty breakfast buffet (provided)

8:30-10:30  Quality and End-Use and Safety/ Qualité et sécurité:
Chairs: David Kaminski and Penny Pearse

8:30  The malting and brewing perspective on FHB in malting barley.
Erin Armstrong (BMBRI, Winnipeg MB)

9:00  Strategies to Enhance Utilization of Fusarium-Contaminated Grains for the Livestock Industry.
James House (University of Manitoba, Winnipeg MB)

9:30  A high throughput optical system for the detection and removal of FDK from Fusarium-infected wheat.
David Prystupa (Spectrum Scientific, Pinawa MB)

10:00 Effects of electron beam irradiation on deoxynivalenol in distillers dried grain, solubles and in production intermediates.
Terry Stepanik (Acsion Industries, Pinawa MB)

10:30-11:00  Refreshment break

11:00-12:00  Wrap-up: Discussion of progress since the 3rd CWFHB
Chairs: Andy Tekauz and Jeannie Gilbert

End of conference
List of Oral Presentation Abstracts and Summaries

**Plenary Session/ Plénière**

29. Diversity of *Fusarium* in Canada: unexpected species and their significance for molecular diagnostics of grain pathogens. *Keith Seifert*

30. Strategies for improving fusarium head blight (FHB) resistance in European wheat. *Thomas Miedaner*

35. Funding Fusarium research: the farmer’s perspective. *Bill Toews*

36. FHB and the bioethanol industry: what is needed? *Anita Brulé-Babel*

**Mycotoxins/ Mycotoxines**

36. Recombinant antibodies with affinity for deoxynivalenol: a novel tool against fusarium head blight. *Patrick Doyle*

37. Challenges for control and certification of mycotoxins in grain shipments: Canadian experiences with ochratoxin A and their possible implications for Fusarium toxins. *Tom Nowicki*

44. Where we should be after three Fusarium disasters in 20 years - 1986, 1996, 2006. *Art Schaafsma*

44. Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* isolates in wheat - results of field, climate chamber, and laboratory studies. *Susanne Vogelgsang*

**Resistance Breeding/ Amélioration de la Résistance**

45. Association mapping in barley for FHB resistance. *François Belzile*

46. The application of marker-assisted selection for breeding FHB resistant wheat in Canada. *Gavin Humphreys*

46. Progress in Fusarium Head Blight Resistance Breeding in Canadian Durum Wheat. *Curtis Pozniak*

47. Progress in breeding for FHB/DON resistance in malting barley. *Linnea Skoglund*
Physiology of Resistance/ Physiologie de la Résistance

50. Finding resistance to fusarium head blight (FHB) in unexpected places. Steve Haber

51. An improved strategy for breeding FHB-resistant wheat must include type 1 resistance. Akos Mesterhazy

66. Metabolomics as a tool to decipher the mechanisms and for high throughput screening of wheat resistance to FHB. Ajjamada Kushalappa

Genomics and Genetics/ Génomique et Génétique

75. Rapid identification of genes contributing to FHB resistance in wheat. Steve Scofield

75. Regulation of the trichothecene pathway in Fusarium graminearum. Gopal Subramanian

76. Targeting scab with defence regulatory genes. Jyoti Shah

76. Proteome and transcriptome analysis of the impact of trichothecenes and Fusarium graminearum in barley. François Eudes

77. The search for QTLs to employ in marker assisted selection (MAS) for FHB resistance in barley. Kevin Smith

78. New insights into the biochemical nature of fusarium head blight resistance using proteomics. Chris Rampitsch

78. GLK1, a novel transcription factor that confers Fusarium graminearum resistance to Arabidopsis: implications to FHB resistance in wheat. Jas Singh

Epidemiology and Disease Management/ Epidémiologie et Contrôle

79. Dry Heat Disinfection versus Seed Borne Fusarium – Six Years’ Practical Experience. Dave Gehl
87. FHB Epidemic risk forecasting system: A Northern Great Plains perspective. Char Hollingsworth (Sent an abstract, and will be sending a longer version)

89. Integrated strategies for FHB management, a Northern Great Plains perspective. Marcia McMullen

94. Risk reduction strategies for FHB and DON contamination in a high moisture environment. Richard Martin

95. Meteorological-based systems for predicting and managing Fusarium head blight epidemics in the wheat growing area of Argentina. Ricardo Moschini

Quality and End-Use Safety/Qualité et sécurité

102. The malting and brewing perspective on FHB in malting barley. Erin Armstrong

103. Strategies to Enhance Utilization of Fusarium-Contaminated Grains for the Livestock Industry. James House

103. A high throughput optical system for the detection and removal of FDK from Fusarium-infected wheat. David Prystupa

104. Effects of electron beam irradiation on deoxynivalenol in distillers dried grain, solubles and in production intermediates. Terry Stepanik

After 20 years of relative stability, *Fusarium* taxonomy is again on the move. The number of accepted species is increasing at an accelerating rate, and the identification manuals most widely used by plant pathologists and plant breeders are badly out of date. Most molecular phylogenetic studies to date have focussed on sections *Discolor* (including the *F. graminearum* complex), *Liseola* (including the *F. verticillioides* complex), *Elegans* (*F. oxysporum* complex) and *Martiella* (*F. solani* complex). As the phylogenetic structure of *Fusarium* in the broad sense clarifies, numerous lineages emerge that have not been comprehensively sampled for species diversity. Only a few species remain readily identifiable by micromorphology alone, notably *F. sporotrichioides* and *F. poae*. For some species, such as *F. pseudograminearum* and *F. graminearum*, culture characters are more helpful for identification than micromorphology of macroconidia. Macroconidial dimensions and shapes change according to the presence or absence of light, especially in species of section *Discolor*, where the classical morphological species concepts are based primarily on these structures. Problems continue over the correct application of names for some species, such as the unfortunate adoption of the poorly typified name *F. cerealis* over the well-typified name *F. crookwellense*, especially in Europe.

How many *Fusarium* species occur in Canada? The idea of about fifteen common species is no longer tenable. Poorly documented species, such as *F. coeruleum* in potatoes, are common. ‘Wild’ species, unassociated with agriculture and often associated with teleomorphs in *Cosmospora*, are also frequent if one knows where to look. Unusual species are sometimes associated with unusual hosts, such as invasive weeds.

We will review recent literature on the taxonomy of *F. graminearum*, which includes the description of additional phylogenetic species, mycotoxin and pathogenicity data for some lineages, and analyses of allele frequency that challenge the acceptance of some lineages as biological species. We will present some of our data using additional genes chosen from genome projects potential phylogenetic markers and present preliminary results on the development of robust real time PCR assays for some grain *Fusarium* species. Because the reliability of such assays relies heavily on adequate sampling of the sequence divergence with a target species and its relatives, we will emphasize the importance of comprehensive phylogenetic sampling as a critical component of the development of molecular diagnostics.
Strategies for improving Fusarium head blight (FHB) resistance in European winter wheat. T. Miedaner. *Universitaet Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, D-70593 Stuttgart, Germany*

Fusarium head blight (FHB) of wheat is a major threat to sustainable wheat production worldwide. In the European Union (EU), wheat is grown on 24.8 million ha with a mean grain yield of 50.9 dt ha\(^{-1}\) in 2006. In the UK and Germany, grain yield reached 80.4 and 72.0 dt ha\(^{-1}\) on average in 2006, respectively. *Fusarium graminearum* is the predominant species causing FHB, however, *F. culmorum*, *F. avenaceum*, and *F. poae* also occur in considerable frequencies. Severe yield losses and contamination by mycotoxins in grain, especially deoxynivalenol (DON), but also nivalenol and zearalenone, that are deleterious to animals and to humans are the result of FHB epidemics favoured by high humidity during flowering, maize as precrop, and reduced tillage. Growing resistant cultivars is the best means to reduce the threat of mycotoxin contamination of cereal feed and food. The latter is a major concern in the EU. For human consumption threshold levels were introduced in 2006 with the following limits: 1750 \(\mu\)g DON/kg in oats, durum wheat, and maize, 1250 \(\mu\)g DON/kg in all other unprocessed cereals, 750 \(\mu\)g DON/kg in flour, 500 \(\mu\)g DON/kg in bread, and 200 \(\mu\)g DON/kg in baby foods.

France, Germany and the UK are by far the largest producers of winter wheat in the EU. Traditionally, varieties from the UK are extremely high yielding, but moderately to highly susceptible to FHB. In France several more resistant varieties are available. In Germany, varieties with susceptibility scores from 2 to 7 on a 1-9 scale (1=healthy, 9=100% infected plot) are available. Varieties with scores 2 to 3 have a market share of 19%, those with 2 to 4 of 65%.

Genetic variation for FHB resistance in wheat was described in several gene pools. Resistance to FHB is of quantitative, oligo- or multigenic nature (Snijders 1990) and, therefore, it is a tedious and time-consuming task for plant breeders to develop cultivars adapted to local environmental conditions with a high level of FHB resistance. In spring wheat, a few highly effective loci were found in exotic stocks from China, especially ‘Sumai 3’ and its derivatives (Anderson et al. 2001, Buerstmayr et al. 2003). In European winter wheat, the genetic basis seems to be more complex (see Table 1), but can be improved by effective (recurrent) selection procedures. For both gene pools, quantitative trait loci (QTL) and linked markers have been identified. To enhance and accelerate progress from selection in developing FHB-resistant wheat germplasm three basic strategies are possible:

1. Phenotypic selection within adapted germplasm
2. Marker-assisted selection (MAS) and pyramidisation of QTLs from adapted European resistance sources
3. Marker-assisted backcross (MAB) breeding of QTLs from exotic resistance sources

**Phenotypic selection within adapted germplasm**

Phenotypic selection is already practised by many commercial wheat breeding companies in Europe. It includes the inoculation of segregating progeny starting with the F\(_4\)
generation or doubled-haploid lines and selection for FHB resistance, often with an independent culling point of rating class 5-6 as favored by the ‘Bundessortenamt’ in Germany. Inoculation is done by spraying conidia onto wheat heads at several dates during flowering or by spreading maize stubble from the previous year in the plots. Both methods are successful, the latter, however, depends more on high humidity before and at flowering and allows a higher influence of morphological characters like plant height.

Estimation of quantitative-genetic parameters in unselected F2–derived lines in the F4 or F5 generation from five crosses with ninety-five progeny per cross showed that the midparent values generally resembled the means of their progeny (Miedaner et al. 2006a). Significant (P < 0.01) genotypic variance was detected within each of the five crosses, but genotype x environment interaction and error variances were also high. Medium to high entry-mean heritabilities (0.6–0.8) underline the feasibility of selecting F2–derived bulks on a plot basis across environments (locations, years). Estimates of expected selection gain are encouraging for breeders to improve FHB resistance by phenotypic selection within adapted materials. In a parallel study, phenotypic correlation between FHB severity and DON content was high (r=0.8, P=0.01; Miedaner et al. 2003). Selection for lower grain DON content and FHB resistance can be effectively started as early as in the F3 generation when bulks are used and tested across several environments. For selection among single plants of the F2 generation, heritability estimates are too low (Snijders 1990). Lines with low DON content can be achieved indirectly by selecting for reduced head blight severity across environments. This was confirmed recently by a selection experiment among spring and winter wheat (Wilde and Miedaner 2006).

A major drawback is a negative correlation between FHB resistance and plant height in many European materials. One reason might be the use of semi-dwarf genes, like Rht-B1, Rht-D1 or Rht 8, that have an impact on FHB resistance. In the UK, the great majority of varieties and in Germany about half of the registered varieties contain the Rht-D1b allele. Progeny with this allele are shorter, but have a considerably higher FHB susceptibility than progeny of the same crosses with the wild-type allele Rht-D1a (Voss et al. 2006). Similar observations were made among progeny of a small Arina x Riband cross (Draeger et al. 2007). Additionally, loci for FHB resistance are often localized near QTLs for tallness in European wheat material (e.g. Schmolke et al. 2005, Häberle et al. 2007). To gain for short-strawed, more FHB resistant varieties by phenotypic selection, progeny from crosses with parents containing the Rht-D1b allele should be assessed for their Rht allele by molecular markers and within the semi-dwarf subpopulation a strict phenotypic selection for FHB resistance should be done. This strategy will increase selection intensity and consequently needs larger population sizes, but should be successful on the long run.

Marker-assisted selection (MAS) and pyramidisation of QTLs from adapted European resistance sources

The use of marker techniques is now feasible since more and more QTLs for FHB resistance have been detected in diverse mapping populations and SSR markers are easy to handle. Basic pre-requisites for their use in practical breeding programmes are the validation of mapped QTLs in independent genetic backgrounds and the enrichment of
QTL regions by informative markers. After that, QTLs can be introgressed into breeding populations and MAS can be performed in the first segregating generation to enrich the population for beneficial QTL alleles.

FHB resistance of European winter wheat sources is obviously governed by several loci with small to medium effects (Table 1). Two of them have been validated recently in the same background as the mapping population (Häberle et al. 2007), but their additive effect in a different genetic background amounted to 3 to 7% only (Korzun et al. 2006). Effects for FHB resistance of 11% were achieved with two QTL combined. The importance of such verification studies is illustrated by the fact that in three populations with the Swiss variety ‘Arina’ no congruency of QTL could be found (Table 1).

Table 1. Mapping results from European winter wheat donors: Number (#) of environmentally stable QTLs for FHB symptoms and percentage of explained phenotypic variance ($R^2$) for individual loci

<table>
<thead>
<tr>
<th>Donor x susceptible</th>
<th># QTL</th>
<th>$R^2$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cansas x Ritmo</td>
<td>7</td>
<td>8-20</td>
<td>Klahr et al. 2004</td>
</tr>
<tr>
<td>Dream x Lynx</td>
<td>3</td>
<td>10-24</td>
<td>Schmolke et al. 2005</td>
</tr>
<tr>
<td>G16-92 x Hussar</td>
<td>2</td>
<td>8-19</td>
<td>Schmolke 2005</td>
</tr>
<tr>
<td>Renan x Récital</td>
<td>3</td>
<td>5-19</td>
<td>Gervais et al. 2003</td>
</tr>
<tr>
<td>F201-R x Patterson</td>
<td>4</td>
<td>2-19</td>
<td>Shen et al. 2003</td>
</tr>
<tr>
<td>Arina x Forno</td>
<td>3</td>
<td>6-22</td>
<td>Paillard et al. 2004</td>
</tr>
<tr>
<td>Arina x NK93604</td>
<td>4</td>
<td>15-28</td>
<td>Semagn et al. 2007</td>
</tr>
<tr>
<td>Arina x Riband</td>
<td>1$^1$</td>
<td>-$^1$</td>
<td>Draeger et al. 2007</td>
</tr>
</tbody>
</table>

$^1$ Out of 8 QTLs only the $Rht-D1a$ allele was found to be significant at more than one location and accounted for 13-24% of phenotypic variance for FHB resistance on four locations; no mean value is given in the paper.

A crucial question for the breeder is, whether he achieves a higher selection gain for phenotypic vs. marker-based selection. We analysed a double cross where three FHB-resistance QTL alleles were introgressed in an elite wheat background. The QTLs derived from ‘Dream’ ($Qfhs.lfl-6AL$, $Qfhs.lfl-7BS$) and ‘G16-92’ (Chromosome 2BL) and a population of 600 lines was selected by one SSR marker per QTL and by phenotypic selection (Miedaner et al., unpubl.). The mean realised response from selection per year was 2.1 vs. 2.5% for the phenotypic vs. marker variant, respectively. MAS is clearly faster and cheaper when selection is restricted to one or two markers of the target QTLs. However, no selection for other agronomic traits is feasible unless verified QTLs for these traits are available. This is especially crucial for plant height, because several major QTLs for FHB resistance are linked with QTLs for straw length. Accordingly, the marker variant resulted in significantly taller progeny than the phenotypic variant in our study. Considering the large genetic variation for FHB resistance within the marker-selected
progeny, a phenotypic selection in the field should follow to exploit the full range of quantitative variation for resistance caused by genes that have so far been undetected in QTL-mapping studies and to select for other agronomic traits.

Marker-assisted backcross breeding of QTLs from exotic resistance sources

The Chinese resistance source ‘Sumai 3’ and its derivatives still have a higher resistance and environmental stability than all tested European winter wheats. The main problem of their use in the high-yielding wheat production areas in Northwestern Europe is their inferiority for grain yield, lodging tolerance, and other disease resistances. Marker-assisted backcrossing might be a solution. The effects of two QTLs of the ‘Sumai 3’-derivative ‘CM 82036’ (Buerstmayr et al. 2003) and the 3A QTL from ‘Frontana’ (Steiner et al. 2004) were validated in an independent European elite background (Fig. 1). They were estimated to be 10% reduction in FHB rating for each of the 3BS (syn. *Fhb1*) and 5A QTL (syn. *Qfhs.ifa-5A*) and 5% reduction for the 3A QTL (Miedaner et al. 2006b). These values are considerably less than those estimated in the mapping population. The mean realised response from selection per year was 3.2 vs. 4.4% for the phenotypic vs. marker variant, respectively, after one cycle of phenotypic and marker selection for the two mentioned QTLs (Wilde et al. 2007).

Obviously, the two exotic donor-QTL alleles had a considerably higher effect than those from European winter wheat. They additionally reduced DON content from 24.9 mg kg⁻¹ in the unselected source population to 7.8 mg kg⁻¹ in the variant with the two QTLs. The best progeny had a resistance and DON content similar to the most resistant parent ‘CM82036’. In a backcross procedure it would, of course, suffice to select progeny with the beneficial donor QTL in the heterozygous state and to produce backcrosses until verifying the effects by phenotypic selection. Using these effective QTL from exotic sources in commercial wheat breeding programmes still requires breeders to estimate their potential side effects on other agronomic traits (linkage

![Fig. 1. Effect of the three donor-QTL alleles on chromosomes 3BS (*Fhb1*), 5A (*Qfhs.ifa-5A*), and 3A, on *Fusarium* head blight rating (FHB) in the mapping and validation populations tested across four locations. Effect = Effect of changing the allele at the respective QTL from susceptible to resistant](image-url)
drag), and to dissect the QTLs into smaller genomic fragments to reduce unwanted effects.

In conclusion, resistance donors and SSR markers linked to verified QTLs for FHB resistance are now available that allow an efficient selection for FHB resistance and reduced DON content. In the short run, phenotypic selection within adapted European wheat has already resulted in fairly resistant cultivars. To improve the performance of high yielding, but highly susceptible cultivars rapidly to an acceptable level of FHB resistance, it might be worthwhile to introgress highly effective resistance-QTL alleles from ‘Sumai 3’ or derivatives by MAB. Then, a background selection for the genome of the elite parent should be considered. In future, near-isogenic lines with shorter QTL segments or even the underlying genes should be available.

References


Miedaner, T., Schneider, B., and Geiger, H. H., 2003: Deoxynivalenol (DON) content and Fusarium head blight resistance in segregating populations of winter rye and winter wheat. Crop Science 43, 519-526

Miedaner, T., Schneider, B., and Oettler, G., 2006a: Means and variances for Fusarium head blight resistance of F-2-derived bulks from winter triticale and winter wheat crosses. Euphytica 152, 405-411

Miedaner, T., Wilde, F., Steiner, B., Buerstmayr, H., Korzun, V., and Ebmeyer, E., 2006b: Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. Theoretical and Applied Genetics 112, 562-569


Wilde, F. and Miedaner, T., 2006: Selection for Fusarium head blight resistance in early generations reduces the deoxynivalenol (DON) content in grain of winter and spring wheat. Plant Breeding 125, 96-98


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**Funding Fusarium research: the farmer’s perspective.** Bill Toews. *Canadian Wheat Board, 423 Main St., Winnipeg, Manitoba, Canada*

The presentation will attempt to view the fusarium issue through the eyes of a farmer. It will provide statistical information on fusarium control measures in cereals taken by farmers and estimate costs for control. Progress in fusarium resistance seen through the farmers eyes will be presented. Farmers' views of funding research will be covered and farmer funding mechanisms will be examined. As well, an overview of other funding mechanisms will be provided.
FHB and the bioethanol industry: what is needed? A.L. Brûlé-Babel. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada.

Wheat and corn are the primary feedstocks for the bioethanol industry in Canada. Fusarium damaged kernels have lower test weight and starch content compared to non-infected kernels and reduce ethanol yields. Mycotoxins in the feedstock also limit the utility and marketability of the dried distillers grains and solubles (DDGS) co-product. Approximately one third of a tonne of DDGS are produced for every tonne of grain processed for bioethanol production. Therefore, mycotoxin concentrations in DDGS are approximately three times higher than in the original feedstocks. Grain buying specifications for the ethanol industry include minimum standards for test weight and maximum tolerances for mycotoxin concentrations. Development of Fusarium head blight (FHB) resistant cultivars would help to ensure a reliable supply of feedstock for bioethanol industry and should be a breeding target for the starch-based bioethanol industry. Lower feedstock costs of FHB infected grain could offset lower ethanol yields if the DDGS could be marketed and utilized. Therefore, development of treatments that degrade mycotoxins to non-toxic components prior to or during fermentation, or that could be applied to DDGS to improve their potential utilization would enable the bioethanol industry to use Fusarium infected grain in years when there are large quantities of low cost FHB infected grain. Further research is required.

Mycotoxins/ Mycotoxines

Recombinant Antibodies with Affinity for Deoxynivalenol: A novel tool against Fusarium Head Blight. P.J. Doyle1, M. Arbabi-Ghahroudi2, N. Gaudette2, A. Hermans3, L. Musa3, G.S. Furzer1, M.E. Savard3, S.C. Gleddie3, C.R. MacKenzie2, and J.C. Hall1. 1 Department of Environmental Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1. Institute for Biological Sciences. National Research Council of Canada. 100 Sussex Drive, Ottawa, ON, Canada, K1A 0R6. 3 Eastern Cereals and Oilseeds Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, Canada, K1A 0C6.

Deoxynivalenol (DON) is a low molecular weight trichothecene mycotoxin commonly associated with Fusarium Head Blight (FHB) infection of cereals and maize crops. Mycotoxin accumulation within FHB-affected grain represents a serious economic problem as, once formed, DON is likely to persist throughout the food chain from harvest to storage to handling to processing of food and feed. DON-binding recombinant single domain (sdAb) and single-chain variable fragment (scFv) antibody fragments were raised through phage-display techniques based on leukocyte RNA isolated from hyper-immunized llama and mice, respectively. After isolation and purification of soluble protein, sdAb and scFv recombinant Ab fragments were tested for binding affinity to DON and structurally-similar trichothecenes by Enzyme-Linked ImmunoSorbent Assay (ELISA) fluorescence polarization and surface plasmon resonance formats. High affinity recombinant antibody fragments were tested for the ability to bind and limit the cytotoxicity of target mycotoxins within Saccharomyces cerevisiae, as a
novel \textit{in vivo} eukaryotic test system. Aside from their standard application within mycotoxin detection systems, we confirmed that recombinant antibody fragments with affinity for DON can bind to and limit cytotoxic effects within eukaryotic cells. A next step for candidate rAbs would be to test their ability to bind to DON within FHB infected plants and thereby potentially limit disease progression.

\textbf{Keywords} Fusarium Head Blight, Deoxynivalenol, mycotoxin, ELISA, antibody fragments, sdAb, scFv, phage display.

\textbf{Challenges for management and certification of mycotoxins in grain shipments:} Canadian experiences with ochratoxin A and their possible implications for \textit{Fusarium} toxins. T. W. Nowicki, Program Manager-Grain Safety Assurance. \textit{Canadian Grain Commission, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8}

Today, grain exporters face heavy demands from foreign customers for reassurance about food safety matters. Providing reassurances to foreign buyers is relatively easy for toxic substances that do not occur in grain or occur only infrequently. However, for mycotoxins which occur frequently in grains and which may occasionally be present at concentrations that approach or exceed accepted standards, reassuring foreign buyers and health authorities is a major challenge. For problematic mycotoxins, we require the means to not only provide basic assurances, but also manage levels and certify the concentrations present in cargoes.

\textbf{Background Issues}

A number of background factors related to deoxynivalenol (DON) and ochratoxin A (OTA) have cropped up since 2000 that have increased the need to manage and certify levels of these mycotoxins in export cargoes. Two recent incidents involving OTA have played a large part in accelerating Canada’s efforts to develop mycotoxin management and certification schemes. Both incidents involved shipments of Canadian durum wheat to Europe. Both incidents involved allegations that the shipments identified contained OTA at levels above the 5.0 ppb standard set by the European Commission (EC). All the cargoes were eventually found to be compliant and the allegations were dropped, but the reputation of Canadian wheat was tarnished.

As a result of these incidents, the Canadian Grain Commission (CGC) and Canadian Wheat Board (CWB) undertook measures to reassure European importers and health officials that Canadian wheat cargoes will be able to meet EC OTA standards and to further international trade. These measures included implementation of a comprehensive OTA management scheme for wheat shipments to the European Union (EU) and application to the EC for approval of pre-export checks for OTA to partially offset the need to test cargoes upon their arrival in Europe.
**Mycotoxin management**

Management of OTA levels in commercial shipments requires the ability to prevent mycotoxin development and/or control mycotoxin levels in bulk cargo shipments. Realistically, the only two possibilities for OTA prevention are: development of strategies and guidelines for grain production and storage and development of chemical treatments for control of fungal infections. The former requires a solid understanding of OTA contamination of Canadian grains. In order to further this understanding, a baseline study of OTA contamination on the Canadian prairies was carried out in 2004-2005 and an OTA grain storage study has been initiated involving Agriculture and Agri-food Canada (AAFC), the CGC, the University of Manitoba and the CWB.

At present, we must rely on control measures to ensure that export cargoes are able to meet customer specifications. Currently, segregation schemes offer our only practical control measures for managing OTA levels in cargo shipments. OTA segregation poses many challenges. The more obvious challenges are the number of analyses that are required and meeting the required turnaround times for results.

**Cargo certification**

Certification of cargo shipments for mycotoxin content presents challenges both on the planning front to develop a strategy for this type of service and on the operational front with respect to analytical testing and issue of the certificates of analysis.

The cargo certification program set up by the CGC for ochratoxin A in wheat cargo shipments was set up early in 2006. The three overriding challenges for the CGC in developing a strategy for an OTA cargo certification service were the need for the service to be operationally feasible, the need for the service to be scientifically credible and the desirability of aligning our certification scheme, wherever possible, with EU sampling and analysis protocols.

**Challenges for analytical testing**

The analytical testing process presents both operational challenges and scientific challenges. The major scientific challenges are related to the difficulty of generating accurate and repeatable OTA test results. All three phases of the analytical testing process: sampling, sample preparation and chemical analysis present challenges in this respect and contribute to the total variance ($V_{Total}$) of an OTA determination.

$$V_{Total} = V_{Sampling} + V_{Sample preparation} + V_{Chemical analysis}$$

There are four factors that contribute to inaccurate OTA results and poor repeatability: (1) the fact that OTA is the by-product of microbiological activity, (2) the fact that OTA contamination in bulk grain is nonhomogeneous, (3) the nugget effect - small percentage of kernels with high OTA concentrations, and (4) the need to measure OTA at parts per billion (ppb) levels.

The difficulty for obtaining accurate and repeatable results at ppm and low ppb levels is evident from the Horwitz equation which states that the percent relative standard deviation (%RSD) among laboratories doubles for every decrease of 2 orders of
magnitude in concentration. At 5 ppb, the predicted repeatability among laboratories is around 35%.

**Sampling**

Sampling is recognized as the largest source of error for measurement of mycotoxins at ppb levels in a bulk lot. For aflatoxin measurements in peanuts, sampling has been reported to account for up to 90% of the total variance of test results.

In Canada, official sampling of grain exports is performed using automatic samplers installed in the terminal and transfer elevators. Samples are taken at the time of vessel loading. EU sampling protocols for control of mycotoxins require analysis of cargo shipments over 1500 metric tonnes (MT) on a 500 MT subplot basis for which 100 incremental samples, roughly 100g each, are taken and then combined to produce a 10 kg aggregate sample. At present, it is not operationally feasible for the CGC to base an OTA certification scheme for wheat shipments on testing of 500 MT incremental loading samples. Instead, cargo certification is based on the official cargo composite sample.

**Sample Preparation**

Conventional sample preparation processes involve passing the entire composite sample through a sample divider to split off the required weight of laboratory sample, grinding the entire laboratory sample, mixing the ground material and subsampling the mixed ground material to obtain the test portion – the amount required for the test method. For OTA analysis, subsampling of whole grain can result in very poor repeatability between replicate laboratory samples from the same composite sample.

Table 1. Affect of subsampling on repeatability for composite samples with two different OTA concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ppb OTA</td>
<td>2.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean %RSD</td>
<td>143.6</td>
<td>47.2</td>
</tr>
</tbody>
</table>

We observed the affect of subsampling on repeatability in a study involving two 20 kg samples of durum wheat containing low levels of OTA. Each sample was mixed and passed through a sample divider to split out ten 2 kg subsamples. For each sample, five subsamples were ground on a Romer mill at its maximum setting and the other five were ground on a LM3100 grinder with a 800 micron screen installed. Each ground sample was mixed and twenty 100g test portions were removed and analyzed for OTA using a modified version of the Vicam immunoaffinity column fluorometry method. The mean OTA concentration and %RSD were calculated for each 2 kg portion of the two 20 kg samples. For the two 20 kg samples, the average mean OTA concentration and %RSD of the mean OTA results over the ten 2 kg portions are shown in Table 1. The results suggest that there is a dramatic decrease in repeatability of results with decreasing OTA concentration in the low ppb range.
Figure 1. Subsampling study - affect of laboratory sample weight (as a percent of the weight of the composite sample) on repeatability for two different composite samples.

For each of the two 20 kg samples, theoretical calculations were made to determine the average mean OTA concentration and %RSD for the means for all the possible combinations of the ten 2 kg samples for producing 4, 6, 8 and 10 kg subsamples - 20 to 50% of the weight of the original sample. Figure 1 shows how the %RSD increases as the relative size of the subsample decreases for samples with two different OTA concentrations. These results show that for a sample with a mean OTA concentration of 3.7 ppb, in order to reduce subsampling error below 40%, the whole grain subsample must weigh about one-third or more of the weight of the composite sample.

Figure 2. Affect of OTA concentration on repeatability of OTA results for five different laboratory sample weights.

Looking at the data in a different way for the 2 kg laboratory sample results and the theoretical results for all the combinations for producing subsamples weighing 4, 6, 8 and
10 kg, we see a sharp rise in %RSD as the OTA concentration drops below 5 ppb and an improvement in %RSD with increasing weight of the laboratory sample (Figure 2).

We observed the effect of whole grain subsampling on OTA results in our ongoing analyses for testing multiple 2 kg laboratory samples from selected cargo composite samples - which initially weigh about 20-30 kg. The analyses were carried out in duplicate or triplicate. The results are shown in Table 2.

Table 2. Repeatability of OTA measurements for testing multiple 2 kg subsamples from a 20 – 30 kg sample.

<table>
<thead>
<tr>
<th>N (samples)</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of 2 kg subsamples</td>
<td>2 - 4</td>
</tr>
<tr>
<td>ppb OTA</td>
<td>1.4 – 5.6</td>
</tr>
<tr>
<td>Mean %RSD</td>
<td>101.0%</td>
</tr>
</tbody>
</table>

By plotting %RSD vs OTA concentration for the individual composite samples, we see an inverse relationship between OTA concentration and %RSD (Figure 3).

These results confirm the conclusion drawn from our sample handling study that a 2 kg subsample from a 20 to 30 kg composite sample is not a sufficient amount of sample for subsampling whole grain.

Subsampling of ground grain for producing the test portion also contributes to inaccurate results and poor repeatability and the coarser the grind the greater the error. If a sample is only coarsely ground, the repeatability of results for replicate test portions will be very poor, even if the ground material is well mixed, unless the relative size of the test portion is very large.
Figure 4. Repeatability of OTA results for one to five 100g test portions (T) subsampled from 2 kg ground wheat (ground using a LM3100 grinder).

Analyzing more than one test portion improves the repeatability of the final result (Figure 4). Five replicate analyses will improve the %RSD to about one-third of what it is for a single determination. Three replicates cuts the %RSD about in half compared to single determinations.

Figure 5. Relationship between %RSD and OTA concentration for two different grinders.

Figure 5 shows how the repeatability of results increases with decreasing OTA concentration for use of the Romer Mill (set to gives the smallest particle sizes possible) and the Laboratory Mill 3100 grinder (with a 800 micron screen). The LM3100 grinder reduces the wheat to a particle size so that 100% of the ground grain will pass through the screen that is installed. The results show much improved repeatability with the LM3100 grinder.

Overall, CGC studies show that the three key factors affecting the variability of OTA results for subsampling ground grain are: particle size of the grind, weight of the test portion relative to the weight of the laboratory sample and number of replicate test portions analyzed.
**Alternative approaches to sample preparation**

European scientists developed the slurry method as an alternative to conventional sample preparation in order to avoid ground grain subsampling errors. The method involves homogenizing a 10 kg sample with 10 kg water and then removing aliquots of the slurry for chemical analysis. The slurry method is reported to achieve %RSDs of less than 10%, but isn’t a practical approach for the CGC to employ in its regional laboratories.

The new innovation is the VSSD – variable split sample divider, from Malvern Engineering. It is reported to grind, mix and subsample in one operation. It looks promising, but is currently unproven for OTA determinations in wheat.

The CGC has developed an OTA method that is essentially a compromise between the conventional approach to sample preparation and the EU slurry method. The method is based on use of a 10 kg laboratory sample and a 1 kg test sample. The entire 10 kg laboratory sample is ground on a LM3100 grinder and the ground grain is mixed on a rotary mixer. A 1 kg test sample is removed and the entire 1 kg is homogenized with extraction solvent. Three aliquots of the extract are carried through the remainder to the chemical method. A method validation study showed a mean %RSD of 9.4%, which is close to %RSDs being reported for the slurry method.

**Chemical analysis**

As far as scientific challenges for the chemical analysis method are concerned, there are background type challenges and challenges related to the methods themselves. One of the main background challenges relates to EU legislation that defines method performance criteria for mycotoxin analysis of food for official control purposes. These criteria weighed heavily in the development of the CGC OTA method.

Three types of methods are commonly used today for OTA measurements: enzyme linked immunosorbent assay (ELISA), liquid chromatography (LC) and immunoaffinity column with fluorometry (IA-FL).

One of the biggest challenges for the CGC was to find a method that is both scientifically sound and practical for use in the CGC’s regional laboratories. ELISA did not meet our requirements in terms of method performance. To date, we have been employing an LC method. The method that we settled on for the long term is the immunoaffinity column-fluorometry method. The method is a modification of the Vicam IA-FL method for OTA in wheat. With one of the modifications to the Vicam method being extraction of a 1 kg test portion, one of the main challenges for use of this method is dealing with the solvent/grain waste. The method has been validated for use at the CGC.

**Conclusions**

Mycotoxin management and cargo certification presents many challenges. The challenges that we faced for OTA were a learning experience in all respects. Having had this experience, we are well prepared to meet future challenges with respect to DON.
Epidemics of *Fusarium graminearum* impact both the corn and wheat industries in SW Ontario. In 1986, SW Ontario experienced a severe epidemic of *F. graminearum* in corn. The immediate reaction was to identify the key factors that contributed to the epidemic, and these were concluded to be in order of importance: weather, genotype, and to a lesser extent agronomy. Many studies within the past 20 years have shown, agronomic factors play a very minor role in *F. graminearum* epidemics when compared to environment by genotype interactions. Spotty outbreaks of Gibberella ear mold occur annually, particularly in the SW regions surrounded by the Great Lakes, and clearly the Swine Industry has borne the greatest impact. Several large projects in the last 20 years were funded to improve corn genotypes, none of which have resulted in material gain in managing the risk of Gibberella ear mold on farms today. In 2006, a much worse epidemic was encountered in corn, and those hybrids with the greatest market share in the affected region were among the most susceptible. In fact, it could be argued that hybrids grown in 2006 were as susceptible as those grown in 1986, because similar levels of deoxynivalenol were recorded in the 5 to 30 ppm range in grain coming from this region in both epidemics. These levels certainly impacted the feed trade, particularly the swine industry, but also the newly emerging industrial use sector, namely ethanol and corn syrup. In contrast, after the last major Fusarium epidemic in 1996 in wheat, the wheat industry has made significant progress in developing and utilizing more tolerant genotypes of wheat, in developing and the deployment of forecasting models, and in developing fungicide strategies. Both industries continue to struggle with inadequate surveillance technology. This paper discusses the reasons why corn has fallen behind and wheat has jumped ahead in the management of Fusarium and its mycotoxins in Canada. It also offers what key steps need to be taken.

Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* isolates in wheat - results of field, climate chamber, and laboratory studies. S. Vogelgsang, M. Sulyok, R. Schuhmacher, and H. R. Forrer. Research Station Agroscope Reckenholz-Tänikon ART, Reckenholzstrasse 191, 8046 Zurich, Switzerland; (M.S., R.S.) Center for Analytical Chemistry, Department of Agrobiology (IFA-Tulln), University of Natural Resources and Applied Life Sciences, Konrad Lorenz Strasse 20, 3430 Tulln, Austria.

The cereal disease Fusarium head blight (FHB) is caused by a complex of *Fusarium* species. Compared with mycotoxins produced by *F. graminearum* (e.g. deoxynivalenol), some toxins of *F. poae* (e.g. nivalenol, diacetoxyseirpenol, T-2) and of *F. avenaceum* (e.g. moniliformin) display a far greater toxicity. This could have significant implications on human and animal health. Moreover, since plants in the field and harvested grains infected by *F. poae* or *F. avenaceum* lack symptoms as distinct as those observed from *F. graminearum*, contaminated cereal lots may be overlooked.
In a 3-year field experiment, we observed highly significant differences in the susceptibility of 14 winter wheat varieties to mixtures of three isolates each of *F. poae* and *F. avenaceum* with incidence on grains ranging from 6 to 49% and from 69 to 92%, respectively. For *F. poae*, a strong correlation between fungal incidence and nivalenol content ($r^2 = 0.89$) was found. In a climate chamber experiment on the spring wheat cultivar Apogee, we found substantial differences in pathogenicity between individual isolates. In an *in vitro* study where the same isolates were incubated on different cereal substrates, we observed strong substrate effects as well as isolate-specific substrate effects on the amount and type of toxins detected.

**Resistance Breeding/ Amélioration de la Résistance**

**Association mapping in barley for FHB resistance.** F. Belzile, S. Marchand and L. Zhang. Département de phytologie, Pavillon Marchand, Université Laval, Québec QC G1A 0A6 Canada.

Association mapping (AM) is a novel genetic mapping approach that is particularly attractive because it allows one to map genes or QTLs in breeding lines and cultivars using phenotypic datasets that are often readily available. It is only recently, however, that the high throughput genotyping technologies required to carry out AM have become available in barley. We are exploring the potential of AM in barley with the aim of identifying genetic determinants for FHB resistance and important agronomic traits. To validate the mapping approach, a preliminary analysis was conducted on plant height and test weight, two traits for which conventional QTL mapping has been conducted extensively. As reported in detail in the poster presented by Zhang et al., a very good level of coincidence was observed between the QTLs detected by the two mapping approaches. To map FHB resistance QTLs, a collection of 68 lines were tested in four nurseries (Brandon MB, Ottawa ON, St-Hyacinthe QC and Charlottetown PEI) in both 2006 and 2007. This collection includes cultivars, breeding lines and sources of FHB resistance currently being used by breeders. DON levels for the 2006 trial have been used to perform AM on this set of lines. The results of this analysis will be presented and discussed at the workshop.
The application of marker-assisted selection for breeding FHB resistant wheat in Canada. G. Humphreys, D. Somers, S. Fox, R. DePauw, R. Knox, F. Eudes and L. Tamburic-Illincic. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9; (R.D., R.K.) Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, PO Box 1030, Swift Current, SK, S9H 3X2; (F. E.) Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, Alberta, T1J 4B1; (L. T-I.) Dept. of Plant Agriculture, Univ. of Guelph, Ridgetown Campus, ON, Canada.

Fusarium head blight (FHB) is a destructive fungal disease of wheat that annually results in economic losses for producers as well as reduced feed and end-use quality for the wheat industry. Development of FHB resistant cultivars is challenging because FHB resistance is controlled by multiple genes, the expression of which is often affected by the environment. Thus, it is difficult to construct and phenotype FHB resistant lines. Much of the most effective genetic resistance has been sourced from unadapted germplasm with linkage drag of undesirable traits. Molecular breeding strategies have been used to develop new breeding materials that combine resistance genes from multiple sources in improved backgrounds. Fine mapping of FHB resistance genes (fhb1 and fhb2) has facilitated screening for FHB resistance in parental lines, in vitro selection, backcross and doubled haploid breeding. High throughput screening technologies such as DNA extraction robotics and multi-channel capillary electrophoresis permit the screening of multiple markers. Wheat breeding lines are regularly screened for FHB resistance loci on chromosomes 3BS, 5A, 6BS and 2D. Haplotype analyses of breeding materials at FHB resistance loci permit selection of resistant lines for advancement and crossing. Future marker-assisted selection efforts will focus on traits which reduce deoxynivalenol content, and the mapping and deployment of non-Asian FHB resistance.

Progress in Fusarium Head Blight Resistance Breeding in Canadian Durum Wheat.
C. J. Pozniak, J. M. Clarke, A.K. Singh, J. Thomas, and G. Fedak. CJP: Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8; JMC and AKS: Agriculture and Agri-Food Canada, Semi-arid Prairie Agriculture Research Centre, Swift Current, SK, Canada, S9H 3X2; JT Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, MB, Canada, R3T-2M9; GF: Agriculture and Agri-Food Canada, Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, Ontario K1A 0C6.

Durum wheat (Triticum turgidum L. var. durum) is particularly sensitive to Fusarium graminearum Schwabe, the causal agent for Fusarium head blight (FHB), and progress in breeding for FHB resistance has been hindered by a lack of resistance sources. Transfer of major FHB resistance quantitative trait loci (QTL) from hexaploid wheat (T. aestivum L.) using molecular markers has had limited success, with decreasing resistance in each successive backcross to durum wheat. Fortunately, improved tolerance has been identified in adapted durum wheat material in Canadian durum wheat programs. Advanced breeding lines derived from DT696, a line developed at Agriculture and Agri-
Food Canada, Semiarid Prairie Agriculture Research Centre, have shown improved
tolerance to FHB in both field and greenhouse testing relative to current check varieties.
Two populations have been developed and efforts to map field resistance are on-going.
In addition, durum wheat has a large number of tetraploid relatives, which represent a
gene pool for improvement of FHB resistance in durum. Resistance from *T. dicoccoides*
and *T. carthlicum* have been identified and efforts are underway to transfer this resistance
to adapted durum wheat breeding lines. A population derived from crossing a *T. dicoccoides*
source with a DT696 derivative has been developed to pyramid resistance
from both sources and is currently being assessed to identify molecular markers to speed
introgression. In the case of *T. carthlicum*, greenhouse resistance has been genetically
mapped and QTL for field resistance are being confirmed in existing mapping
populations and near-isogenic pairs.

**Progress in Breeding for FHB/DON Resistance in Malting Barley.** Linnea G.
Skoglund. *Busch Agricultural Resource, Inc., Fort Collins, CO USA*

Progress in breeding for FHB resistance in US malting barley has been painfully slow.
After fourteen years of breeding effort, new cultivars and elite breeding lines are
available that reduce DON accumulations by up to 50%. To get to this point has required
collaboration of the spring barley breeders, pathologists and other barley scientists. This
collaboration has lead to 1) sharing of malting barley germplasm with minor genes for
resistance, 2) identification of new sources of resistance, 3) establishment of nurseries for
disease screening, and 4) increased capacity and decreased cost for DON testing.

**Sources of Resistance**

Legacy, released in 2000, was the first BARI cultivar to accumulate significantly less
DON than commercially available cultivars. The BARI breeding program now has many
lines in its elite and parent germplasm that reduce DON accumulation by 30-50% of
Robust. This progress is the result of incremental integration of genes available within
the BARI germplasm as well as UM and NDSU germplasm. Overall, this approach has
been a matter of integrating multiple genes with minor effects into an elite line that has
acceptable malt quality – not an easy task.

Other sources of resistance have been identified from the National Small Grains
Collection (NSGC), the ICARDA/CIMMYT breeding program, Composite lines
(originally from Ramage), Swiss landraces, the Vavilov Collection in Russia and others.
Projects are currently underway at various universities to determine the genetic diversity
of several of these. Meanwhile, many are in use by BARI.

Screening of the NSGC was done at BARI Seed Research from 1998-2001 and at NDSU
from 1999-2001. Over 8100 6 rowed spring barley accessions were screened by one or
both groups. Screening was carried out in the field and in the greenhouse. The BARI
group identified 15 accessions for possible breeding and the NDSU group identified 10.
Only one accession, CIho 6613 (Seed Stocks 1148-1), was identified by both groups (Skoglund and Menert, 2002; Steffenson and Scholz, 2001). Three NSGC accessions (Canadian Lake Shore, 1948D and Seed Stocks 1148-1) were crossed to Legacy in 2000. Though several selections from the Legacy/Seed Stocks 1148-1 cross did well, they failed to advance through our selection process.

Collaboration with the ICARDA/CIMMYT barley breeding program has facilitated the exchange of resistant germplasm and crossing with elite malting lines. There is the added advantage of multiple disease resistance. A number of lines have been crossed once or twice to BARI elite lines or cultivars and are now in various stages of testing. F2s of crosses made in 2007 are planted in a winter nursery at Davis, CA and will undergo screening in the greenhouse at BARI. Crosses from 2004 and 2005 were planted as F6 headrows in summer 2007. Selections from these currently are planted in the off-season nursery in China. Following is a list of resistant parents.

- Ataco/Bermejo//Higo/3/CLN/Gloria/Copal/4/Chevron
- Chamico
- Canela/Zherdar#2
- Madre Selva
- Gob/Humai10//Canela/3/Aleli
- Arupo/K8755//Mora/3/Gob/ Humai10/4/Shyri
- Canela
- Tocte//Gob/Humai10/3/Atah92/Aleli
- Svanhals-Bar/MSel/Azaf/Gob24DH
- Penco/Chevron

Dr. Brian Steffenson (University of Minnesota) has been instrumental in identifying and distributing sources of resistance from other, less accessible sources. These include Composite Cross XXX, Swiss landraces, the Vavilov Collection in Russia and Nordic Gene Bank (Steffenson, 2003; Steffenson and Dahl, 2003; Steffenson et al, 2005). Below is a list of the sources used by BARI and their current status.

<table>
<thead>
<tr>
<th>ACCESSIONS</th>
<th>TYPE</th>
<th>YEAR</th>
<th>SOURCE</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMP 351</td>
<td>6 rowed</td>
<td>2003</td>
<td>Composite Cross</td>
<td>F7 in China</td>
</tr>
<tr>
<td>COMP 355</td>
<td>6 rowed</td>
<td>2003</td>
<td>Composite Cross</td>
<td>F7 in China</td>
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<tr>
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<td>2 rowed</td>
<td>2003</td>
<td>Swiss Landrace</td>
<td>Crossing block</td>
</tr>
<tr>
<td>HV 531</td>
<td>2 rowed</td>
<td>2003</td>
<td>Swiss Landrace</td>
<td>Dropped*</td>
</tr>
<tr>
<td>VIR 20738</td>
<td>6 rowed</td>
<td>2004</td>
<td>Vavilov Collection</td>
<td>Dropped*</td>
</tr>
<tr>
<td>VIR 20733</td>
<td>2 rowed</td>
<td>2004</td>
<td>Vavilov Collection</td>
<td>Dropped*</td>
</tr>
<tr>
<td>VIR 16537</td>
<td>2 rowed</td>
<td>2007</td>
<td>Vavilov Collection</td>
<td>Crossing block</td>
</tr>
<tr>
<td>VIR 28797</td>
<td>6 rowed</td>
<td>2007</td>
<td>Vavilov Collection</td>
<td>Crossing block</td>
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<tr>
<td>VIR 28807</td>
<td>2 rowed</td>
<td>2007</td>
<td>Vavilov Collection</td>
<td>Crossing block</td>
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<tr>
<td>NGB 9443</td>
<td>6 rowed</td>
<td>2007</td>
<td>Nordic Gene Bank</td>
<td>Crossing block</td>
</tr>
</tbody>
</table>

*Recommendation from B. Steffenson after further testing.
FHB Nurseries and Collaborative Screening

We participate in a number of misted, inoculated nurseries and collaborative trials around the world. The inoculated nurseries have proven invaluable as insurance against low DON years in our yield trials. The North American Barley Scab Evaluation Nursery (NABSEN) is an international screening nursery that includes six breeding programs and is planted in 8-10 locations, both dryland and irrigated. The Mississippi Valley Barley Nursery and the Midwest Coop are collaborative trials made up of elite lines from a number of breeding programs. The trials are grown by multiple collaborators in multiple locations. Some collaborators evaluate these for resistance to FHB and DON.

DON Testing

For the past 8-10 years, we have placed high priority on screening for DON accumulation in the BARI breeding program. This has been facilitated by the establishment and/or expansion of a number of facilities with funding from the US Wheat and Barley Scab Initiative. Also, BARI Seed Research has collaborated with Dr. Nick Hill, Agrinostics Inc, in testing an ELISA-based technique for quantifying *Fusarium graminearum* mycelium in grain.

Conclusion

Malting barley breeding programs are making progress. There have been incremental improvements in DON accumulation in advanced lines using a variety of genetic resources. These lines are slowly advancing through the testing and acceptance procedures on their way to farmers’ fields and the brew house. ND20448 (NDSU) and M122 (UM) have made it through American Malting Barley Association approval process and will be tested in brewing trials by Anheuser Busch in 2008.

REFERENCES


**Physiology of Resistance/ Physiologie de la Résistance**

**Finding resistance to fusarium head blight (FHB) in unexpected places.** Steve Haber, S. Golkari and J. Gilbert. *Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg MB R3T 2M9, Canada.*

Wheat that combines high quality and yield with FHB resistance similar to ‘Sumai 3’ remains an elusive target. With several, or perhaps many, genes involved in expressing the trait, it has proved difficult to retain high FHB resistance while advancing through cycles of crosses with elite, FHB-susceptible parents. Recent work has shown that the expression of genes in resistance pathways induced by *Fusarium* inoculation does not differ significantly among ‘Sumai 3’ and FHB-susceptible near-isogenic lines (NILs). We reasoned commercial cultivars might similarly already contain resistance genes induced by *Fusarium* but remain susceptible because, in contrast to ‘Sumai 3’, their expression was not coordinated effectively. In succeeding generations under pressure from early-growth-stage inoculation with wheat streak mosaic virus (WSMV), ‘McKenzie’, a doubled-haploid (DH) spring wheat cultivar, gives rise to variant sub-lines that differ heritably from type with respect to height, maturity and resistance to WSMV. An array of such sub-lines was evaluated in an FHB nursery in 2007 and lines with FHB resistance clearly better and others clearly worse than the original ‘McKenzie’ were identified. A modified version of this approach applied to successive backcross cycles with the FHB-susceptible durum cultivar ‘Strongfield’ as the recurrent parent has generated lines with excellent agronomic traits and FHB resistance similar to ‘Sumai 3’. 
An improved strategy for breeding FHB resistant wheat must include Type I resistance. Mesterházy, Á.1; Buerstmayr, H.2; Tóth, B.1; Lehoczki-Krsjak, Sz.1, Szabó-Hevér, Á1, Lemmens, M.2.1 \textit{Cereal Research non-profit Co., Szeged, Hungary}; 2 IFA Tulln, Austria.

Abstract

Breeding programs in FHB resistance concentrated mostly on Type II resistance in USA, Canada, and China, but in Europe a lot of spray inoculation is used and selection for sure combines Type I and II (plus other components). Increasing experience shows that materials containing the 3BS QTL from different sources do not have stable high resistance; plants that are more susceptible can also be obtained. Therefore, the need to introduce more intensively also the Type I resistance became obvious. Results show that the Type I resistance as a response to spray inoculation is confusing. Spraying inoculation covers both Type I and II and makes the identification of further resistance components possible. It seems that some methodological variations of the spraying inoculation and use of contaminated grain help get closer to the genetic nature of resistance. We think now that with spray inoculation we measure the contribution of Type I and II resistance components to FHB resistance, and that with point inoculation we measure mainly type II resistance. Type I is not directly measured, but we can calculate indirectly its effect when resistance data obtained after spray inoculation are compared with resistance data obtained with point inoculation. Data clearly show that the Sumai 3-derived 3BS for Type II and 5A for Type I have the same significance. Both code alone a medium level of resistance, but highly significant differences exist within groups. They influence not only FHB, but also the whole disease process including toxin response. As 5A and other Type I QTLs were not analyzed in most breeding programs, the significance of 3BS was overemphasized. This seems one of the major reasons of the sometimes disappointing progress. As the use of small and medium level QTLs is so far poorly supported, their application in breeding programs is questionable at present. FHB QTLs are race-non-specific and species-non-specific.

Introduction

The FHB, caused by \textit{Fusarium} spp., mostly \textit{F. graminearum} is the most destructive disease of wheat as it causes not only significant yield losses, but deteriorates the remaining yield by a number of toxins with very different chemical properties and effects on humans and animals. Following devastating epidemics, an intensive breeding work started in all continents. The most important efforts in resistance research were made in China, Europe, US and Canada; smaller programs are operating in many countries. The breeding work concentrated mostly on the 3BS QTL and markers were often used to identify superior genotypes. Two basic screening were used, the point inoculation method for Type II and different versions of spraying inoculation as well as infected grains (kernel spawn method) served as source of infection, and both combined
with some art of mist irrigation. However, most breeding program relies on the Type II resistance, as it is easy to do in greenhouse and the results are highly repeatable (Griffey 2005, Anon. 2007, Dill-Macky 2003). Recent results clearly show that this led to a significant progress, but Type II plants often have rather different degrees of susceptibility, which contradicts the assumption that Type II is effective enough to solve alone the breeding problems. It seems the time is here to reconsider some clichés we often use, but now do not seem fully adequate to the new tasks to be solved. The need is not new, Comeau et al. (2003) outlined some emerging problems, and now the situation is ripened to summarize what we have and maybe new insights will also help to modify some views and research tools to find a more effective way to solve problems.

In the contribution we analyze now what resistance components have been described, what is their content and how they can be measured. We summarize the knowledge on the QTLs, what function they may play in resistance expression. Also important to define which trait seems to be more reliable to estimate resistance. One must ponder how the large, medium and small effect QTLs should be treated in a breeding project. At last, some conclusions will be drawn and we would like to outline possibly more effective ways to select highly resistant wheat genotypes.

Components of resistance.

Type I and Type II resistance were described by Schroeder and Christensen (1963). Type I is the resistance component against initial infection or invasion. No precise description was given, but Dill-Macky (2003) considers that type and underlying mechanisms of resistance are rarely understood and that the isolation of the Type II resistance in field nurseries originated largely from difficulties in preventing multiple infection events in field-grown plants. Steffenson (2003) stresses the significance of Type II resistance and mentions the spraying inoculation, which identifies Type 1 resistance in sensu Schroeder and Christensen (1963). This means that Schroeder and Christensen considered the plant response to spraying inoculation as defining Type 1 resistance. Mesterházy also used that definition (Mesterházy 2003). Gilchrist et al (1997) found a correlation between the two types of resistance indicating some similar parts of underlying mechanisms. Ban (1997) thought that the initial infection means a resistance mechanism at the very beginning of the infection process, but only small differences in penetration rates were found between resistant and susceptible genotypes. Steiner et al. (2004) considers Type 1 resistance to be equal with the ratio of infected heads in the whole plot, irrespectively of the severity of the infection within head. However, it depends on ecology, so a clear description is hardly possible. Additionally, methodical problems also inhibit classification of resistance types (Engle et al. 2003). Therefore there is no true agreement in the definition of Type 1 resistance. When we would like to use Type 1 resistance, we should have a clear definition, which describes the background more precisely than what we accepted until now. For the nomenclature of the other three types we may go back to Mesterházy (1995), because thereafter the different components were mentioned in different order (see also Mesterházy et al., 1999). Resistance to kernel
infection (Type 3) and tolerance (Type 4) were described later followed by resistance to DON (Type 5). The latter seems to be a complex trait with several aspects like degradation of DON. For more information on this, the reader is referred to Abramson et al. (1998), Lemmens et al. (2005), Miller et al., (1985), Mesterházy 2002, Mesterházy et al. (2005). Stack et al. (2005) confirmed the existence of type 4.

Until now molecular genetics did not help much to clarify the story. It is not an accident that Type II is preferred and actually nearly all molecular genetic work except several papers support this Type. Type 1 QTLs were described at present in Europe, the 5A QTL from Sumai 3 is considered Type 1 and the Frontana has Type 1 resistance as it is highly susceptible to the point inoculation (Steiner et al 2004, Buerstmayr et al. 2003). Both large (5A) and small to medium effect QTL were identified, and the situation is the same for Type II resistance, too. In Frontana, for example, a small degree of Type II resistance was found (Steiner et al. 2004). It is a common experience that in both cases only visual head symptoms are analyzed and additional traits like FDK, DON or tolerance were not considered, even though the DON and other toxins provide the largest problems as a result of infection. We have excellent information about the genetic background and regulation of toxin production of the pathogen but only sporadic information is at hand about what happened in the plants. This is very important, since the resistance plays a significantly higher role in toxin regulation than the ability of the fungal strain to produce toxins (Mesterházy et al. 2005).

Methodology

In the Szeged research program we worked mostly by the spraying inoculation, with a rather labor intensive methodology without mist irrigation. FDK, yield and toxin are investigated since 30 years (Mesterházy 1995, 1998, Mesterházy et al. 2006). In Tulln an art of spraying inoculation comined with mist irrigation was used (Steiner et al 2004). Although point inoculation would have been easier, we concentrated to develop a spraying methodology with good accuracy and repeatability as it is relatively independent from morphology traits, like plant height differences. Even more important, it mimics the natural infection where spores land on the outer surface of the ear and do not fly directly into the ovary of the floret.

Results

Multiresistance against different Fusarium spp.

In the first experimental series we tested genotypes with different Fusarium spp. (Figure 1). It is clear that the resistance is active against all Fusarium spp. tested. The amount of disease depends on both aggressiveness of the isolate and resistance of the plant as well as environmental factors, and resistance is much more important in
regulation of the epidemics than the disease-causing ability of the fungus. On the most resistant wheat lines, even the most aggressive isolates cause only sporadic and low level symptoms. The most resistant materials have 3BS QTL, however the Type 1 Frontana and Arina gave also good performance, although they were more infected; especially at high disease pressure those could suffer considerable infection (Mesterházy et al. 2005). The DON values (Fig. 2) from the same test carry an important message. The aggressiveness of the DON producing isolates is proportional with the FDK data; e.g., the toxin producing ability of the isolates is dependent on their aggressiveness. However, on highly resistant materials even the most aggressive and highest toxin-producing isolates can produce only very low toxin amount.

Although on the most susceptible genotypes the most aggressive isolate can produce DON up to 400 ppm, DON contamination is very low in the most resistant ones. It is important that DON reduction in Type I and Type II genotypes is proportional with the symptom severity. Therefore, the hypothesis can be formulated that both resistance types have similar role in symptom and DON inhibition. We suppose that both Type I and Type II resistance will, in the end, reduce DON contamination, but with a different mechanism. How these mechanisms differ was not well understood. It now seems that Type I acts more on the fungus, thus reducing DON contamination, while Type II acts on the toxin (detoxification) resulting in an indirect effect on the fungus. However, new findings may modify this view. Fig. 3 shows the mean reactions of the genotypes across isolates for the four traits considered. Highly resistant genotypes have both type I and II. In the first group (right side of the figure) the genotypes are about 10 days earlier than the late western European wheats at the left of the figure, and the high 60-80 % FDK corresponds with 40-50 ppm DON. In the late materials, the DON production is 2-5 times higher. From our point of view, the conclusion is that somehow, the longer vegetation period allows higher DON accumulation. The data were confirmed also by tests in 2004 (unpublished). Recent tests show that among western European materials resistant materials also exist. Based on this, we proposed that cultivars should not be registered without resistance and toxicology screening.

To see the function of the different QTLs we have tested a set of genotypes (24 lines with 3BS, 24 with 5A, 24 with both and 24 lines with no known QTL) of the DH population CM82036/Remus made in IFA Tulln, Austria. The lines were tested in Austria and Hungary by different spraying inoculation methods over two years against eight isolates of different Fusarium spp. including an isolate of Microdochium nivale. Evaluation was made for many traits including FHB, FDK, yield, and DON contamination. Of all disease traits, the FDK was the most stable one across years and locations and here was received the largest QTL effect. Table 1 shows the general means for wheat lines and isolates.

When present alone, the 3BS (Type II) and 5A (Type I) can provide only a medium level of resistance (Table 1). The positive effect on FHB resistance of each QTL is similar in magnitude, decreasing the disease severity by about 40 %. A real significant decrease is observed when the two types of the resistance act together; this synergetic effect provides nearly 75 % reduction in FDK. This means for us that the 3BS QTL alone
does not provide sufficient protection and may explain the inadequate resistance level often observed in 3BS QTL progenies. It is important that the QTLs are effective against the different Fusarium spp. The non-species-specific activity of these two QTLs was demonstrated, correlations between resistance data towards different Fusarium spp. were highly significant ($r = 0.97-0.99$).
Table 1. QTL group means for the FDK values across years and locations, 2002-2004

<table>
<thead>
<tr>
<th>QTL groups</th>
<th>Check</th>
<th>Fg12377</th>
<th>Fg44</th>
<th>Fc12375</th>
<th>Fc89.4</th>
<th>Fav-38</th>
<th>Fspor</th>
<th>FavIFA60</th>
<th>Mniv 3/98</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>3B/5A</td>
<td>0.19</td>
<td>2.37</td>
<td>1.07</td>
<td>9.48</td>
<td>14.14</td>
<td>16.91</td>
<td>3.42</td>
<td>7.05</td>
<td>1.39</td>
<td>6.22</td>
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<td>3BS</td>
<td>2.11</td>
<td>13.79</td>
<td>4.20</td>
<td>27.47</td>
<td>33.91</td>
<td>39.71</td>
<td>9.16</td>
<td>16.34</td>
<td>6.04</td>
<td>16.97</td>
</tr>
<tr>
<td>5A</td>
<td>0.51</td>
<td>8.47</td>
<td>4.20</td>
<td>17.39</td>
<td>32.60</td>
<td>41.07</td>
<td>8.29</td>
<td>16.62</td>
<td>6.25</td>
<td>15.04</td>
</tr>
<tr>
<td>No QTL</td>
<td>2.27</td>
<td>20.10</td>
<td>11.93</td>
<td>35.60</td>
<td>47.73</td>
<td>56.33</td>
<td>17.58</td>
<td>24.96</td>
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<tr>
<td>Mean</td>
<td>1.27</td>
<td>11.18</td>
<td>5.35</td>
<td>22.49</td>
<td>32.10</td>
<td>38.50</td>
<td>9.61</td>
<td>16.24</td>
<td>6.81</td>
<td>15.95</td>
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<tr>
<td>LSD 5 %</td>
<td>1.23</td>
<td>4.4</td>
<td>3.01</td>
<td>5.01</td>
<td>5.19</td>
<td>5.08</td>
<td>1.24</td>
<td>3.35</td>
<td>2.58</td>
<td>3.03</td>
</tr>
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</table>

(Fg= F. graminearum, Fc= F. culmorum, Fav= F. avenaceum, Fspor= F. sporotrichioides, Mniv= Microdochium nivale)
Fig. 4. Distribution of the DH lines within QTL groups for FDK (%) across years, locations and isolates, 2002-2004 (LSD 5 % between any genotype is 3.28)

The story will be even clearer in Figure 4. In each genotype group, we have a large variation in % FDK, and the differences between maximum and minimum values exceed nearly ten times the LSD 5 % limit value. Here we can ask the question: what is really the strength of the known and unknown QTLs in terms of explaining the total behavior. It is possible to hypothesize the existence of different susceptibility QTLs or other uncommon genetic mechanisms. If we took as baseline value the mean of the 3-4 most susceptible genotypes within each group, the resistance improvements observed should be determined by unknown QTLs. This variation is observed in all 4 groups showing that additional resistance factors distributes nearly evenly in all genetic groups studied. The most resistant member of the no QTL group is actually better than several genotypes with two QTLs. We think this goes along very well with the fact that the single 3BS QTL genotypes may respond as medium or more susceptible to FHB. This is supported by the fact that, in Arina and Frontana, at least 8-10 medium or low effective QTLs were identified, thus a similar situation could be realistic also for CM 82036, a Sumai-3 (Sumai 3 /Thunderbird) derived line. A QTL on 6B has been identified for Sumai 3, and from traditional monosomic analysis, at least six chromosomes were found to carry some effect on resistance (Buerstmayr et al. 1997, Yu 1982).

We see the same phenomenon for DON also (Fig. 5); this proves that the QTLs do not determine only FHB or FDK, but also DON response.
Resistance to kernel infection, DON accumulation and tolerance were also analyzed. We found that in each group, genotypes were found that had resistance to kernel infection; however, the highest ratio (58%) was in the group with two QTLs. For DON resistance this number was 42% for the 3BS/5A group, and 37.5% for the 3BS. It seems therefore that the 3BS QTL has an influence on the resistance to DON and the mechanism can be the one we have described recently (Lemmens et al. 2005). A mechanism was found that detoxifies DON resulting in the non-toxic DON-3-O-glycoside. The fact, however, that such DON resistant plants were found also in 5A group and the group without these QTL mean that different mechanisms work in the plant, and not only one mechanism. Surely, the Type I and Type II differ in action, but similar mechanisms may also be present.

Correlations between traits.

Breeders often claim that correlations between traits are rather poor (Comeau 2003, Tamburic-Ilicic 2006). This has a number of reasons. Mesterházy (1997) and Mesterházy et al. (2006) discuss some of the methodological problems. We present here the data of the cultivar and line tests from 2006. The correlation between FDK and DON (Figure 6) is very close, even when the inoculation was made with the four isolates in one replicate only for screening. For us the trait is important which shows the closest correlation with DON contamination. In many experiments, the FDK is the best parameter, especially when the flowering period and the inoculation period are long. We had examples when FHB data showed 5-6 times difference for the different inoculation times, but for FDK the variation was much smaller. For FHB and DON correlation, the $r$ value is much lower and this is also the case for the FHB and FDK data (Fig. 7 and 8).
Fig. 6. Genotypes from the FHB program, relation between FDK and DON contamination, means for four isolates, 2006

\[ y = 0.5804x + 1.9926 \]

\[ R^2 = 0.7891 \]

Fig. 7. Regression for the FHB and FDK data in the 2006 FHB resistance tests across four isolates

\[ y = 0.7648x - 0.5257 \]

\[ R^2 = 0.2881 \]
Marker assisted selection.

The QTL analysis has the task not only to identify QTLs, but also markers that co-segregate with the trait. For QTLs with a large effect, the identification is relatively effective, but the situation is completely different for the medium and small effect QTL. Table 2 presents the LOD data for *F. graminearum* isolates according to FHB and FDK in the DH population Frontana/Remus originated from IFA Tulln Austria from the Szeged tests. Inasmuch as we know, this is the first analysis done on FDK values. The problem is that even the best markers give positive signal only in some cases. For example BARC 197 was good in 2002, in 2004 only FDK had a significant LOD value, in 2005 there was no positive answer and in 2006 again there was a full success. Among the chromosomes, the 5A seems to be the most effective. For the medium and low effect QTLs a marker was not yet identified that could provide significant help in selection. The FDK shows also contradictious results, sometimes it has a significant LOD value when FHB does not have a signal and vica versa. Other unknown QTLs may also play a role. We have the same situation in Arina (Draeger et al. 2007), where three papers were published with QTLs, but not much common conclusions between them was found. For this reason, we think that the routine use of these markers is premature.
Table 2. Performance (LOD values) of markers in Frontana/Remus population, Szeged, 2002-2006.

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<td>2.638</td>
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<td>5A BARC 197</td>
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</table>

Fg = F. graminearum, Fc = F. culmorum. Only LOD values above 2.5 are printed.

Breeding aspects

The most important results of the QTL analyses until now is, that they characterize the resistance sources and help to choose the most appropriate parents. In most cases FHB resistance seems to be additive; other combined effects were mentioned, but recessive QTLs were not found until now. So the combinations can be controlled in F1 for FHB resistance and about one third of the crosses can be discarded as was the case in 2007. In F2 and F3 generations, we propose that no inoculation should be made, however from F4 the ear to row selections are exposed to FHB infection. About 10-15 heads will be sprayed and bagged for 48 hrs (one group of head per row without further replicates). FHB scoring will follow three weeks after inoculation. Susceptible lines and others with undesired traits will be discarded. From the selected lines, the infected heads will be harvested and threshed at low wind. After screening for FDK about 50% of the lines will be additionally discarded. This type of screening will be followed until the new lines reach the first yield trial without replicates from the uniform ear-to-rows tests. In subsequent four-replicated field trial four isolates without replicates are used, e.g. the behavior of the lines can be checked in four epidemic situations, so the decision is easier. In the multilocation trial the same work is done in two replicates and this goes further when the line is tested in the variety office. At registration we have at least four years data at hand. Determination of DON will be made each year for the selected lines by HPLC.
The commercial breeding needs in a year about 3-4 thousand ear to row lines to be inoculated and in the advanced generations additionally 1-2 thousand inoculations are needed. Two people can inoculate in the morning about 500 lines, so the work can be managed. In this way, all types of resistance are expressed and selection is made phenotypically. At the end of the selection markers will be used again to check which QTLs secure resistance in the resistant materials. This methodology is used with small modifications since decades. By this way, a number of highly resistant genotypes could be breed, and for a general summary, see Fig. 6.

Discussion

Type I. and Type II. The presented data (Table 1, Figures 4 and 5) clearly show that by the spraying inoculation method both Types of resistance will be expressed, therefore the view that the spraying results would reflect only Type 1 resistance in sensu Schroeder and Christensen seems to be incorrect. It is also clear that a synergetic effect between the two QTLs exist, even their mechanism is unknown. However, this does not mean that such synergetic effect would be valid for other QTL pairs. We think therefore that the spraying methodology is more effective as it helps assay a wider genetic background. The real question is not, which type of QTL do we need. We need both and the selection system we apply supports this need. Another problem is that the resistance types of the selected resistant plants cannot be classified by the spraying methodology, thus, an additional test for Type II resistance will be needed to do this.

The extraordinary role that the 3BS QTL and similar types of QTLs have reached is a consequence of 1) the easy assessment of type II resistance (including a low dependency on environmental factors), 2) a clear definition of this resistance component, 3) the large effect of this QTL (at least within the point inoculation method). However, it seems that Type I resistance is often present in the background, supporting a good resistance expression. As this was not regularly controlled, the first conclusion could easily be that the 3BS is the most important QTL, and that the others might have much smaller role. We now propose that a research program should be started to clarify the genetic background of the 3BS resistant materials, to see whether Type I resistance is present additionally in the background, and how much of the resistance of these materials is really due to Type I vs Type II resistance. For Sumai 3 the 3BS’s role seems to be clear, but behind it, other powerful QTLs may be hidden. In Sumai 3 the two QTLs have an additive effect, as confirmed by Ban and Suenaga (2000). The results until now show that a given genotype contains often both resistance types like Frontana (Steiner et al. 2004); Sumai 3 and many Agropyron accessions had both resistance types (Ban 2000). This points out we need to understand the role of the genetic background.

Multiple resistances to Fusarium spp. It is important that Fusarium resistance is not only race-non- specific, but also species-non-specific (Mesterházy et al. 2005). This means that a single pathogenic isolate is enough to screen for general Fusarium resistance. This is true for the members of the newly described F. graminearum species
complex (Tóth et al. 2007, in press). This explains why a wheat genotype remains resistant against Fusarium head blight in different regions with diverging species composition.

**Screening systems.** The most important lesson is that the screening system must recognize all types of resistance, i.e. not only one. As the spraying inoculation covers both, the screening and resistance work should be based on some art of spraying inoculation or kernel spawn inoculation, with or without misting or bagging. For screening of large populations many inoculation systems work with acceptable results. Sometimes whole fields are sprayed several times, and then mist irrigation is given in different regimes. In other cases a genotype is sprayed only once, or even up to 3-5 inoculations are given, but the mist irrigation follows unevenly, as the earliest materials may receive 2-4 times more water as a two days misting is applied after every treatment. Infected maize and other grains can serve as inoculum as done in many nurseries supported by misting. For a screening, this is also good, but to measure resistance on the field as exact as possible, it is not the most suitable. For the registration, for phenotyping, for other purposes where high preciosity and repeatability is needed, other approaches will also be needed. Therefore we use more isolates so as to create different epidemic situations; do not we inoculate every day, but every 4-5 days so as to minimize the number of inoculations necessary. Even so, the methodology is a critical question, and we have to understand the influence of different methodical regimes on the quality of the data. Different methodologies could be compared on genotypes with different genetic background. Maybe selected members of a DH population could also be used as we did with the CM83036/Remus population. This could help understand the role of the QTLs, and also help decide which methodology gives the best answer.

Analyzing different screening and resistance testing methodologies is beyond the scope of this contribution, but it would be highly desirable to test and compare inoculation techniques. Of course, FDK, and toxin should also be included. We think that those breeding programs which apply some art of spraying or natural-like screening technology are due to be more successful because they deal also with Type I and help accumulate Type I resistance.

**Role of QTLs in screening.** For now, two large effect QTLs are known. One is the Sumai-3 derivated 3BS and the second is on the 5A, again from Sumai-3. The 3BS QTL was detected in many Asiatic resistant materials. Even if a fine mapping experiment found these QTL to be located in the same chromosomal regions as it was done, we are not convinced that the genes are exactly the same. There are several indications that the problem has not been solved. A MAS for these two QTL is possible. However, for all the other published QTL the data is not yet fully convincing. It is not clear whether the QTLs described have really a real contribution to FHB resistance or are only artifacts, which come from the inadequate phenotyping or genotyping or traits that influence FHB expression like flowering time, plant height, etc.. In addition, methodical discrepancies might play a significant role. The validation of the QTLs seldom occurred, therefore before using them a validation in other mapping populations with the same resistance donor would be desirable.
Could a breeder assess his 2000 plants with 20 markers for FHB resistance? It is cheaper to perform a good phenotypic selection under high infection pressure from year to year and at the end check the QTLs. The other argument is that we have strong evidence about unknown QTLs. This can be concluded based on the phenotypic selection.

Another question is: who knows how the different QTLs might cooperate, what interactions may be present as was shown for 3BS and 5A. For this reason the phenotypic selection is more powerful. For the large effect QTLs, however a MAS is now possible. We hope that several other large effect QTLs will be discovered and in this case the significance in practical breeding can increase. When the medium effect QTLs can be convincingly identified, they can also be utilized for MAS. QTL analysis has a further very important task. Determining the position of the QTL in the given chromosome will lead us to the genes and to the cloning the genes. Then the QTLs would become obsolete, but the markers can be developed to the gene itself, and afterwards, FHB resistant plant might be created, transferring the gene directly to adapted cultivars by already known processes.

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References


ABSTRACT

Metabolomics, a technology of identification and quantification of all the metabolites in an organism, is a cutting-edge biochemical tool for functional genomics or for direct application to improve plant trait. Though the plant kingdom is estimated to produce more than 200,000 compounds, a given plant species (Arabidopsis) produces about 5000 metabolites. Several of these are implicated in defense against abiotic and biotic stresses. Using gas chromatography and mass spectrometry we have detected in wheat spikelets several hundred peaks and tentatively identified about 200 components. We have used univariate analysis to identify resistance related (RR) metabolites, where the resistant cultivars and near isogenic lines had higher abundances. Multivariate analysis identified biological functions and the metabolites related to those functions. Several of the RR-metabolites identified here are known for their defense roles as signal molecules, antimicrobial, cell wall enforcing compounds, or intermediate compounds in several metabolic pathways leading to the production of these metabolites. The metabolites detected were related to metabolic pathways and the most active pathways were phenylpropanoid, myoinositol and octadecanoic acid. The technology developed here can
be used to link metabolites to genes in NILs conferring resistance, thus explaining the mechanism of resistance in wheat against FHB.

1. INTRODUCTION

In the post genomic era, metabolomics is gaining an unprecedented importance for its potential to unravel the hidden details about functions of genes. Metabolomics is the technology of identification and quantification of all the metabolites in an organism. Metabolites are the end products of gene expression, and thus, are used in the discovery of genes and explanation of gene functions (8, 13). This technology has been exploited as a tool to discover natural products, drugs, diagnosis of stress, stress resistance, and improve traits (12). In metabolomics, a comprehensive analysis of all the metabolites in an organism, is aimed to better understand the system behavior. However, based on only 74 metabolites the variation in food quality and quantity of tomato has been explained (13). Metabolites have been linked to genomic locations or quantitative trait loci (QTL), and the closely located genes/QTLs regulate specific steps in a metabolic pathway (8). This paper presents the potential of metabolomics as a tool to decipher the mechanisms and develop a metabolomics technology for high throughput screening of resistance in cereals against fusarium head blight. The role of metabolites in plant disease defense is not new. Significant amount of information is already available in the literature about antimicrobial and cell wall reinforcement properties of metabolites. These can be used to putatively explain the mechanisms of resistance (1). Metabolomics, however, is a comprehensive analytical tool that can help put the pieces of the metabolite based resistance puzzle together.

Resistance in plants to pathogens can be true or apparent; in the former the resistance is controlled by genes for resistance which combat the pathogen (its metabolism), but not in the latter which mainly includes disease escape. The true resistance can be preexisting (constitutive) or induced, and each can be structural or biochemical defenses (1). Generally, these, especially the biochemcials, are tissue specific and are located in outer cell layers of plants to defend the pathogen from entry and establishment in the plant. Another grey area is the tolerance, which is the genetic ability of the plant to reduce the damage (as reflected on yield) and not the pathogen (3). The apparent resistance is partly related to plant characteristics (also agronomic) that influence the microclimatic conditions that reduce pathogen inoculation and infection, mainly under field conditions. Plant height, spikelet spacing, etc. can create microclimatic conditions less suitable for infection by the pathogen under field conditions, thus reducing disease progress in field. These are useful traits but care must be taken when used in breeding programs and their further recommendation.

Both true and apparent resistance has been reported in cereals against FHB. The true (active) resistance in cereals is quantitatively expressed and is continuous (2, 10). This can be quantified based on epidemiological parameters such as infection efficiency, latent period, lesion expansion and sporulation (based on monocyclic process), and the rate of disease progress in field (limited polycyclic process). The pathogen effect on plant metabolism also can lead to externally invisible disease symptoms, often not quantified, leading to significant yield losses. Quantitative resistance does not protect the plants becoming infected, but reduces the frequency of infection and also further development of pathogen in the host. In cereals, the true resistance to FHB is quantified based on five
discrete types of resistance (2, 10). The most commonly used type in the selection of breeding lines is the type II because of the repeatability of the test results over years, as opposed to type I which is highly variable, especially under field conditions. The type I is considered to indicate resistance to invasion and type II to disease spread, the latter mainly through detoxification of a virulence factor, deoxynivalenol (DON). However, the proof of mechanisms of resistance discriminating these two types of resistance is yet to come. In extensive screening experiments, cultivars with type II also showed type I resistance, meaning overlapping of resistance mechanisms. This may be because both spray and point inoculations, used to evaluate the type I and type II resistances, respectively, become quite similar in the way they face resistance mechanisms in plant spikelets when inoculated at anthesis. It has been reported that the anthers/reproductive parts enhance pathogen colonization. In the spray inoculation though we expect the pathogen to go through the resistance barriers, the lemma and palea, the time chosen to inoculate at anthesis makes the method ineffective and accordingly it becomes quite similar to type II. Inoculation of spikelets before anthesis (closed florets) can better assess the amount of resistance in outer spikelet layers. In the type II, however, the pathogen is placed in an optimum environment for infection. High type II resistance is considered to have a barrier at the rachis. This is considered to be mainly due to detoxification of DON, as high type II were often associated with low DON content in grains. Thus, further considerations are needed to come up with a better protocol to screen the phenotypes based on disease severity and other indicators of damages caused by pathogen.

Resistance in cereals to FHB depends on genes controlling the traits related to pathogen invasion and further development. This can be better assessed based on resistance mechanisms, which are directly related to genes. These mechanisms can be one or more of the four mechanisms of resistance (constitutive and induced, and in each structural and biochemical), operating from inoculation to spore dissemination, affecting various metabolic functions of the pathogen, leading to reduced levels of disease severity and physiological effects of the host. Though these may involve several specific mechanisms it is possible to identify certain major one and used for breeding. Several genes in a genomic location (QTLs) are known to operate together to perform certain biological function, leading to the production of several metabolites related to a pathway (8). It is possible to identify such QTLs and use them for breeding. In wheat, the evaluation based on type II resistance has been used to identify QTLs. Generally these studies are associated with DON quantification in grains, which is an indicator of mechanism of reduced DON synthesis or DON detoxification. Several QTLs with type II resistance have been identified, and some of these have pleiotropic effects, including apparent resistance (2, 14). The most significant QTL on chromosome 3BS, identified in Sumai3, is associated with an enzyme that conjugates DON, making it less of a virulence factor or pathotoxin. However, resistance to DON explains only about half of the resistance to spread of disease (9), meaning involvement of mechanisms of resistance other than DON detoxification. It is possible that more than one mechanism could be involved in most of the QTLs identified based on point inoculation confirming type II resistance. A few QTLs with type I resistance also has been identified based on spray inoculation. Several breeding lines have been reported to have high type I resistance, such as Duo, Nass, CIMMYT selections, variants of Superb, etc. (Drs. F. Eudes and A. Comeau, personal communications). Though high level of type I is desired to reduce
progress of pathogen under field conditions, thus reducing toxin production, the methodology to evaluate this suffers from disease escape. Breeders are concerned about discarding excellent materials for lack of such a tool. A better understanding of the mechanisms of resistance is crucial to select suitable QTLs, with proof for its intactness for complete functionality, and their further incorporation into elite cultivars. Accordingly, a metabolomics approach was studied to identify the functions of known QTLs, and also to independently develop a metabolomics technology for high throughput screening for resistance. It is hypothesized that the cultivars varying in resistance also vary in their metabolic profiles, and a set of resistance related metabolites (QTLs) can be used as biomarkers to screen for resistance.

2. METABOLOMICS EVALUATION - STEPS

Metabolomics evaluation, comparative analysis of organism metabolism, involves four major steps (Fig. 1): 1) Sample collection; 2) Metabolite extraction; 3) Metabolite analysis using chromatography, including data-output processing to obtain data-sets; 4) Data-set analysis, bioinformatics to extract information, and knowledge generation (6, 7, 11).

2.1 Sample collection: The sample collection depends on the organism and the type of study. In wheat the *F. graminearum* infects spikelets at anthesis. Accordingly the inoculated spikelets after 24 or 48 h of incubation were sampled. Alternatively, the organs such as rachis, rachilla, glume, lemma, palea and reproductive parts can be separately sampled for detailed analysis. Soon after removal the samples were crushed in liquid nitrogen and stored at -80 C until use.

2.2. Metabolite Extraction: Though there is no universal solvent to extract all the metabolites in an organism several hundreds to more than a thousand metabolites have been extracted using organic solvents such as methanol+water (polar metabolites) and chloroform (non-polar metabolites).

2.3. Metabolite analysis using chromatography

Technology platforms: Several technology platforms such as gas chromatography and mass spectrometry (GC/MS), liquid chromatography MS (LC/MS), nuclear magnetic resonance (NMR), etc. are available to analyze the metabolites. However, each has certain advantages and disadvantages, and thus, a combination of technology platforms may be needed for large scale applications. GC/MS is still considered to be the most versatile and popular of all, and typically 300 – 500 peaks can be detected in an analysis or sample. Since nonvolatiles can not be analyzed using GC/MS, other platforms such as LC/MS also has been used. Using accurate mass detection (LC-ESI-QTOF-MS; LC-LTQ-Orbitrap-MS) thousands of metabolites have been identified and related to genes and traits (5, 8, 12).

MS-output analysis: The analysis of outputs from GC/MS (also LC/MS), on scans, molecular masses and abundances, involves the use of several bioinformatics tools, eventually to identify metabolites (6, 7; Fig. 2): i) Peak deconvolution: This is done using AMDIS (Automated Mass spectral Deconvolution and Identification System). This has options to correct for base-line shift that can reduce the background noise, peak separation based on algorithm and selection of metabolites based on peak purity. ii) Compound identification: The compound is identified using the extracted pure mass spectra by comparing it to those in libraries such as NIST (National Institute of Standards
and Technology), GMD (Golm metabolome database; 11), etc.; iii) Peak alignment and abundance: AMDIS gives only ion abundance, and the peak/component alignment and abundance calculation can be done using MET-IDEA (Metabolomics ion-based data extraction algorithm). The abundances are relative concentrations of compounds and the actual concentration can be derived by spiking authentic samples; iv) Data filtration: The dataset can be borrowed into MS-EXCEL and the Pivot Table procedure can be used to delete all the metabolites with poor signal to noise ratio (S/N <20:1 = often leading to poor spectral match), low peak purity (<20%) and inconsistent among replicates. On the other hand the LC/MS with accurate mass can be baseline corrected using MetAlign or other software, and directly related to theoretical masses to putatively identify metabolites (5, 8). Confirmation requires mass fragmentation or other information (NMR, etc.).

2.4. Data analysis, bioinformatics and interpretation of results: Metabolite profile data is not information and information is not knowledge. Information can be generated using univariate and multivariate statistical methods, and also bioinformatic tools. Univariate analysis: has been used to identify the individual metabolites that classify the treatments. The metabolites that are in higher abundances in a resistant cultivar than those in a susceptible cultivar were designated as resistance related (RR) metabolites. RR-metabolites based on water inoculated were designated as constitutive metabolites (RRC) and the ones based on pathogen inoculated were designated as induced metabolites (RRI). These metabolites can be used as biomarkers in high throughput screening of breeding lines.

Multivariate analysis: The dataset on abundances of metabolites has also been subjected to multivariate analysis to classify the treatments, to separate resistant cultivar from susceptible. Principal component analysis, factor analysis (6) and canonical discriminant analyses (7) have been used to classify treatments. A vector (canonical-CAN, principal component-PC or factor-F) that classified the treatments is considered to identify a biological function. The biological function, in turn, can be explained based on metabolites loading highly to that vector. All these analyses give substantial information about the problem, depending on the experimental design. Bioinformatics: The information derived here on metabolite identity (RR-metabolites) has been related to metabolic pathways using several databases, such as KEGG, to identify the metabolites or precursors belonging to a given pathway (Fig. 3). The metabolites can also be further related to proteins and transcripts, and eventually to genes, using several bioinformatics tools. Thus, discovering novel genes related to resistance, a metabolic phenotype or trait, following bottom-up functional genomics approach. Knowledge generation: The information on RR-metabolites, identified following statistical analysis, can be related to information on metabolic pathways, and their putative role in plant defense can be searched in the literature (or proved experimentally), eventually to generate knowledge and to apply this for high throughput screening of resistance.

3. MECHANISMS OF RESISTANCE TO FHB

We have developed metabolic profiles for different combinations of near isogenic lines (NILs) with alternate alleles for a QTL and cultivars varying in resistance, inoculated with *F. graminearum*, DON, or water with objectives to a) putatively explain the mechanism of resistance; b) identify RR-metabolites for potential application as
biomarkers in high throughput screening of breeding lines. Out of several hundreds of analytes/compounds detected in each chromatogram we have putatively identified about 200 metabolites.

**Mechanism of resistance:** Several RR-metabolites were identified. Those identified using NILs with alternate alleles of FHB resistance QTL in chromosome 2DL, for resistance and susceptibility, are considered to explain the mechanism of resistance, as there would be no host genetic background effect. However, the QTL we used has several genes, and finely mapped QTLs should better identify the resistance function. We have identified several RR-metabolites in QTL at chromosome 2DL. The number of RR-metabolites and the pathways in which these were activated were fewer in NILs as compared to Sumai3, and other cultivars in general. Generally, a cultivar has several known QTLs, and it is possible that the different QTLs regulate different metabolites (mainly abundances). Most of the RR-metabolites identified here were constitutive metabolites. These can reduce the rate of pathogen development in the host, reducing in turn the amount of trichothecenes produced. Thus, most of the RRC-metabolites may not only reduce the pathogen development at the outset (type I) but also continue to do so reducing disease progress (affecting all types, including type II). There is evidence that the DON reduction is mainly through enzymatic binding of DON, and the converted product (DON-3-O-glucoside) can be detected using LC/MS (9). Thus, a comprehensive metabolic profiling, of NILs and cultivars, would not only detect the metabolites directly involved in plant defense but also the products of enzymatic reactions, indirectly explaining the mechanisms of resistance.

**RR-metabolites as biomarkers for screening:** We have quantified FHB severity as proportion of spikelets diseased. Epidemiological or quantitative resistance parameters, should better explain the resistance in cereals against FHB. Metabolic profiling studies of several cultivars with high levels of resistance yielded several (close to 50) resistance related metabolites. Their putative mechanisms of resistance were searched in the literature and most had antimicrobial, signaling or plant cell reinforcement properties. In one of our studies involving six wheat cultivars with high levels of resistance, the classification of cultivar resistance to Disease Severity Phenotypes (DSP, based on proportion of spikelets diseased) was not directly correlated with Metabolic Profile Phenotypes (MPP, based on metabolites) (7). This shows that there are several combinations of mechanisms of resistance are involved to arrive at a given level of resistance. Accordingly, we have identified a set of RR-metabolites, with different mode of actions, based on relative comparison to a susceptible cultivar(s) for potential application as biomarkers. However, we have not quantified all the metabolites in wheat spikelets, to explain all mechanisms.

**RR-metabolites and metabolic pathways:** Some of the RR-metabolites identified in our studies are shown in Fig. 3. Even though compound identification is not needed to use a RR-metabolite as a biomarker, it is important in our study to identify and find putative roles of them in plant defense, as the RR-metabolites were not identified using NILs with different QTLs with single genes (8). We have tentatively identified the metabolites using commercial libraries, searched in the literature for possible antimicrobial properties, and also tried to relate them to metabolic pathways to better explain their putative roles in resistance, to use them as biomarkers with more confidence. Some of the important pathways are (7, unpublished data):  

a) Myoinositol
pathway: Myoinositol and its derivatives detected in our study are known to be involved in the cell signaling and pathogen resistant membrane bio-genesis in plants. Inositol oxygenation leads to synthesis of cell wall polysaccharides which make pathogen penetration difficult. Myoinositol and gluconic acids are also precursors of ascorbic acids, an antioxidant that protects plants from pathogen invasion and toxin detoxification; b) Phenylpropanoid pathway: Known to produce several antimicrobial compounds and cell wall barriers, such as lignins. Cinnamic acids and their derivatives such as p-coumaric acid, 3-methyl-cinnamic acid and 4-methyl cinnamic acids were identified as RR-metabolites. Cinnamic and benzoic acids are involved in salicylic acid production, a signal molecule known to activate several PR-proteins, leading to the production of toxin-detoxifying enzymes, and hydrogen peroxide and phenols that inhibit biosynthesis of trichothecenes. Glucaric and galactaric acids form esters that are antimicrobial. Amino acids such as serine and threonine are involved in PR-protein synthesis; several quinones identified here as RR-metabolites are known antimicrobials and trichothecene synthesis inhibitors; c) Polyamine pathway: Polyamine conjugates of cinnamic acids are well known antimicrobial compounds, such as putrescine and spermidine; d) Octadecanoic acid pathway: Octadecanoic acids (stearic acid, linolenic acid) detected here are involved in the synthesis of jasmonic acid that is known to induce several plant defense compounds.

Several metabolites detected in our study are known to detoxify DON and other fungal toxins, in addition to enzymatic detoxification, through oxidation by chemicals such as hydrogen peroxide, ascorbic acid, etc. A pathogen related signal, salicylic acid, is known to activate a multigenic family of glycosyltransferases in the phenylpropanoid pathway. These glucosyltransferases are also involved in the synthesis and assembly of several polysaccharides and lignins, and we have detected high levels of cinnamic acids, p-coumaric acids, benzoic acids, etc. Furthermore, not all the RR-metabolites that were detected in Sumai3 (has several QTLs) were observed (high abundance) in a NIL with FHB-QTL at 2DL. In the latter, the phenylpropanoid pathway was moderately active but the polyamine pathway leading to the production of putrescine, and several others, were not very active. We hypothesize that a comparative metabolomic evaluation of NILs with different single FHB-genes/QTLs at different chromosomes should reveal the functions of these QTLs in wheat for resistance against FHB. Accordingly, it is possible that a list of RR-metabolites or the genes/QTLs controlling these can be accumulated in a cultivar(s) to increase resistance. The RR-metabolites identified here have the potential as biomarkers for high throughput screening of resistance in wheat (cereals) against FHB. Though, metabolic profiling is expensive and time consuming during the stages of biomarker development, the final application protocol can be a target analysis, using RR-metabolite standards, and/or shorter chromatogram run time (about 3 min./sample). For analysis, the samples can be shipped to a central location if equipment is unavailable. The bioinformatics tools can facilitate automation. Since non-volatiles can not be analyzed using GC/MS, currently we are using LC/MS (LC-ESI-LTQ-Orbitrap-MS) for barley-FHB metabolic profiling (5, 8). A preliminary run detected more than 500 components.

In Arabidopsis, colocation of genes/QTLs was associated with coregulation of metabolites related to specific steps in a metabolic pathway, explaining certain biological function (8). It is possible that in Sumai3, the several genes located in QTL at 3BS may be controlling different steps in a metabolic pathway, while the different QTLs control
different satellite metabolic pathways. Identification of RR-metabolites in different NILs with single FHB resistance QTLs based on metabolic profiling can reveal the metabolic pathways regulated by each QTL. Furthermore, finer genomic mapping (4) of QTLs with several genes and their metabolic profiling can explain mechanisms controlled by each gene/QTL. Also it can reveal if any of the RR-metabolites was deleted in the process of fine mapping, reducing the functionality of that QTL, leading to a reduction in the level of quantitative resistance. The use of a gene, with narrow function, in breeding could increase selection pressure and also would be detrimental to biodiversity. Metabolic profiling can reveal several mechanisms of resistance in cereals against FHB and there is potential for its application as a high throughput screening tool.

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Selected references:
Fig. 1. Steps in metabolite profiling (left)
Fig. 2. Steps in GC/MS-output analysis (right)

Fig. 3. Satellite metabolic pathways, of wheat spikelet, by which some of the RR-metabolites are linked (see text).
Rapid identification of genes contributing to FHB resistance in wheat. Steven Scofield. Crop Protection and Pest Control Unit, USDA – Agricultural Research Service, West Lafayette, IN

Development of wheat and barley with improved Fusarium head blight (FHB) resistance will be greatly aided by knowledge of the plant genes that make essential contributions to the FHB resistance mechanism. This knowledge will permit identification of the best naturally occurring variants for use in breeding and identification the genes to be utilized in transgenic solutions to FHB. However, identification of genes contributing to resistance to FHB is greatly hindered by the lack of a rapid assay for gene function in wheat and barley. In this presentation, I will discuss a system we have developed for virus-induced gene silencing (VIGS) which permits the rapid “switching off” of all copies of genes chosen by the experimenter. We are currently employing this system to switch off genes with suspected roles in FHB resistance. If we observe that switching off a candidate gene causes normally resistant plants to become susceptible, we conclude that the candidate has a validated function in FHB resistance.

Regulation of the trichothecene pathway in Fusarium graminearum. G. Subramaniam. ECORC, 960 carling avenue, Ottawa, K1A 0C6, Canada

Activation of the trichothecene pathways in the pathogen Fusarium graminearum results in the accumulation of the mycotoxins DON and its acetylated forms. In the completed genome of Fusarium, both the biosynthetic and regulatory genes for DON are clustered. Three genes Tri6, Tri10 and Tri15 belonging to the Cys2His2 family of Zinc binding proteins play various roles in regulating this pathway. TRI6, with potential transcriptional regulatory functions is required for the expression of trichothecene pathway genes. Mutant lacking a functional TRI6 have greatly reduced levels of pathway gene transcripts leading to drastic reduction the production of DON. The mutant also compromises the virulence of the pathogen. In the current model, TRI6 is proposed to act as a regulator for the trichothecene pathway. This presentation will highlight some of the tools used to further our understanding of Tri6 as transcriptional regulator and a key player in Fusarium pathogenesis.
Targeting Scab with Defense Regulatory Genes. Jyoti Shah, Ragiba Makandar, Vamsi Nalam and Harold N. Trick. Department of Biological Sciences, University of North Texas, Denton, TX 76203, (HNT) Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

Fusarium head blight (FHB), also known as Scab, is a devastating disease of wheat and barley that limits crop productivity and grain quality. *Fusarium graminearum* is the principal causative agent of FHB in North America. We have developed a host-pathogen system consisting of *Arabidopsis thaliana* and *F. graminearum* to identify host mechanisms and genes that contribute to defenses against this fungus. Our long-term goals are to engineer the expression of these defense regulatory genes for enhancing FHB resistance in wheat. Our studies have identified salicylic acid (SA) as an important signaling molecule in plant defense against *F. graminearum*. Furthermore, the *Arabidopsis NPR1* (AtNPR1) gene, which is a key regulator of SA signaling, is a good candidate for enhancing FHB resistance in wheat. Transgenic spring wheat cv. Bobwhite plants engineered to constitutively express AtNPR1, exhibited heightened resistance to *F. graminearum* in greenhouse and growth chamber studies. Furthermore, DON content was lower in the transgenic seeds. AtNPR1-conferred FHB resistance was associated with the faster and stronger activation of SA signaling in the AtNPR1 expressing plants. AtNPR1 expression has also been successfully engineered into durum cultivars. FHB evaluations of these transgenic plants are ongoing. Results on other promising genes identified in our Arabidopsis-*F. graminearum* screen will also be discussed.

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Proteome and transcriptome analysis of the impact of trichothecenes and *Fusarium graminearum* in barley. J. Geddes¹,², B. Selinger³, A. Laroche¹, and F. Eudes¹.¹

Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta, CANADA T1J 4B1. ² University of Lethbridge, Alberta, Canada T1K 3M4

Two-dimensional polyacrylamide gel electrophoresis was performed on proteins from infected heads of six barley genotypes: one susceptible cultivar (Stander), two intermediate resistant cultivars (CDC Bold and Chevron) and three resistant genotypes (CI4196, Harbin and Svansota). Five inoculums, GZT3639, GZT40 (a non-producing trichothecene mutant of wild-type GZT3639), GZT40 supplemented with deoxynivalenol (DON), DON, and water, were used to point inoculate every second spikelet at anthesis. Spikelets were collected for proteome analysis to compare both systemic changes and
constitutive differences in the proteomes of the susceptible and resistant genotypes. Spikelets from Chevron and Stander where also harvested for mRNA extraction and transcriptome analysis using the barley Affymetrix chip. Thirty-three acidic protein spots were differentially expressed three days post-inoculation. Proteins responsive to FHB included those associated with oxidative burst and oxidative stress responses, and defense response (t-test, P<0.05). An increase in abundance of PR-1, PR-3 or PR-5 could be associated with the resistant genotypes, as well as the intermediate resistant genotypes. The RNA microarray investigation revealed a complex cellular network in the barley cells in response to the fungus, the mycotoxin, and the subsequent interaction between them. Both Chevron and Stander appeared to up-regulate gene transcripts associated with the jasmonic acid pathway and showed up-regulation of many gene transcripts coding for PR-proteins (e.g. PR-10, PR-13, and LTPs), but differed in their responses to specific treatments and their induction timing. At least three distinct response patterns are reported from these 6 barley genotypes.


Progress in breeding resistance to Fusarium head blight (FHB) in barley has yet to take advantage of genetic mapping information. Three genomic regions in barley have been identified in multiple studies as containing quantitative trait loci (QTL) for FHB. Each of these regions is associated with a trait that is either undesirable from a breeding perspective or with a trait that can influence and complicate the measurement of resistance (ie. late heading or tall plant height). Exploiting these QTL regions for marker assisted selection (MAS) requires fine mapping and separation of undesirable linkages. In the case of the QTL region on chromosome 2H bin 8, we have identified a recombinant in which a late allele for a heading date QTL has been unlinked from a resistance allele at a QTL for FHB. Similar efforts are in progress for the other two QTL regions. Significant progress has also been made in breeding for resistance without QTL mapping. Supported by the USDA Barley Coordinated Project, we are now collecting data to conduct genome-wide association mapping using breeding germplasm to identify new QTL that are segregating in U.S. breeding germplasm. MAS strategies that combine QTL identified in both bi-parental and genome-wide association mapping should accelerate the development of resistant varieties.

The interaction between Fusarium graminearum and wheat (Triticum aestivum) is being investigated at the biochemical level using two-dimensional gel electrophoresis to analyze differences in the proteomes of wheat lines bearing known, mapped resistance genes. These are: HC374, bearing resistance QTLs 3B (fhb1), 2D, 5A, 6B (fhb2) and 4B; BW278 (fhb2 and 5A); Sumai 3 (fhb1, fhb2 and 5A). In addition three susceptible lines were used, namely, AC Foremost, BW301 and Thatcher. All plants were point-inoculated at anthesis, using Fg or water, and then harvested for analysis after 1, 2 and 3 days. Protein extraction based on acetone/TCA precipitation, and 2DE (IEF x SDS PAGE) was used to visualize their proteomes. Currently a total of 91 differentially expressed protein spots from three parental lines, BW301, HC374 & Sumai 3, have been analyzed by liquid chromatography/mass spectrometry (LCMS) using a linear ion trap tandem mass spectrometer, and identified using Mascot software to query three databases: wheat EST, F. graminearum (full genomic sequence) and NCBInr. The potential role of these proteins in FHB resistance and susceptibility will be discussed.

Segregating populations made from these parents will now be investigated with the same approach to demonstrate segregation of proteins with disease resistance, thus potentially introducing a new biomarker(s) for FHB resistance. Furthermore the biochemical effect of some of these proteins will be assessed in vivo using a viral-induced gene silencing approach (VIGS) in barley.

GLK1, a novel transcription factor that confers Fusarium graminearum resistance to Arabidopsis. Implications to FHB resistance in wheat. L Savitch, G. Allard, G. Subramanian and J. Singh, Eastern Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, ON, K1A 0C6, Canada.

The availability of genomic tools have allowed researchers to identify ‘crosstalk’ or significant overlaps of signalling pathways and networks of gene expression between plant responses to pathogen invasion and to environmental stresses such as drought and cold (Fujita et al., Current Opinion in Plant Biology 2006, 9:436–442). The elucidation of these overlapping networks can identify molecular switches such as transcription factors or kinases that can be exploited to build up the plant’s basal defences to diseases such as those caused by necrotrophic pathogens. Using overexpression followed by transcriptional profiling in Arabidopsis to characterize transcription factors that respond to stresses, we observed that the ‘regulon’ of the GLK1 (Golden2 Like) transcription factor encoded plant disease defence related proteins. GLK1 and 2 transcription factors have been previously implicated to be involved in chloroplast development. Affymetrix Gene Chip and RT-PCR analyses indicated that GLK1 overexpression in Arabidopsis enhanced a high constitutive expression of genes encoding disease defense related proteins such as PR10, isochorismate synthase, antimicrobial peptides, glycosyl
hydrolases, toxin efflux (MATE) and other genes associated with pathogen response and detoxification. Most interestingly, GLK1 overexpression in Arabidopsis confers resistance to *Fusarium graminearum*, a broad host pathogen responsible for major losses in cereal crops. This is the first identification of the GLK1 ‘regulon’ and of a novel role for GLK1 in plant defense, suggesting its potential use for providing disease resistance in crop plants. However, PR1, an indicator of systemic acquired resistance (SAR), WRKY70 and 54, transcription factors responsive to salicylic acid, were downregulated in GLK1 overexpression. This suggests that GLK1 overexpression does not act by conferring a more effective response to pathogen infection but rather by establishing a more robust constitutive basal level of defense and therefore suitable for conferring increased resistance to necrotrophic pathogens such as *Fusarium graminearum* in important crop plants.

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**Epidemiology and Disease Management/ Epidémiologie et Contrôle**

**Dry Heat Disinfection versus Seed Borne *Fusarium* – Six Years’ Practical Experience**

David Gehl. *Agriculture and Agri-Food Canada, Indian Head Research Farm, P.O. Box 760, Indian Head, Saskatchewan, S0G 2K0 (e-mail address: gehl@agr.gc.ca)*

Since 2001 the Agriculture and Agri-Food (AAFC) Seed Increase Unit (SIU) has eradicated *Fusarium graminearum* from >60 tonnes of Breeder seed using a method of dry heat disinfection developed by researchers with the Canadian Grain Commission and AAFC. To the best of the author’s knowledge the SIU is the only pedigreed seed facility in Canada using this technique on a routine basis.

The data in this report is not from replicated scientific experiments but has been collated from legal documents: Certificates of Seed Analysis and Reports of Seed Analysis, which were obtained from accredited seed laboratories for certification purposes. Apart from the calculation of arithmetic means no statistical analyses have been performed on the data.

The AAFC Seed Increase Unit at Indian Head, Saskatchewan produces Breeder seed of AAFC-developed crop varieties. This Breeder seed is distributed to Select seed growers across Canada to initiate the production of pedigreed seed which is, in turn, planted by commercial farmers. The unit’s current inventory comprises 269 varieties of 43 crop kinds including 183 varieties of cereal crops. These cereal varieties constitute a significant portion of commercial grain production in Canada.

The SIU is located in southeastern Saskatchewan where Fusarium Head Blight (FHB) is endemic. Seed borne *Fusarium* in Breeder seed became a major issue for the SIU in the mid 1990’s when it was apparent that FHB was causing major economic losses to grain producers in several areas of Canada and that the causal fungi could be spread by infected seed.
The initial steps to control FHB in Breeder seed produced by the SIU were agronomic practices. Since 1996 these have included: seed treatment, multiple applications of foliar fungicides during the pre-anthesis to soft dough stages, mixtures of contact and systemic fungicides, fungicide application with double nozzles to target the heads of cereal crops, post emergent broadcast application of KCl fertilizer and reduced use of glyphosate herbicide.

Although agronomic practices have reduced FHB in Breeder seed to generally low levels they have been unable to eliminate seed borne infection in years favourable to disease development (Tables 1 & 2).

**Fusarium Status Prior to Seed Disinfection**

Before 2001 Breeder seed of cereals was tested only for the presence of *Fusarium spp.* In 2000, a year favourable to FHB development at Indian Head, all 23 lots of cereal Breeder seed produced by the SIU were positive for *Fusarium spp.* (Table 1). In 2001 conditions were less favourable for FHB still, 29 of 32 cereal lots were positive for *Fusarium spp.* Since 2000, 184 of the 210 retained cereal Breeder seed lots produced by the SIU have been positive for *Fusarium spp.* (Table 1).

In 2001 commercial seed labs in western Canada introduced testing for individual *Fusarium* species including *F. graminearum*. Since then all cereal Breeder seed lots produced by the SIU have been analyzed for both *F. gr.* and “other” *Fusarium spp.* Of the 187 retained cereal Breeder seed lots produced since 2001, 100 have tested positive for *F. graminearum* (Table 2). These 100 *F. gr.*- positive seed lots represent 53.5% of all retained cereal Breeder seed lots and 62.5% of the *F. sp.*- positive lots produced since 2001 (Tables 1 & 2).

**Alberta Fusarium graminearum Management Plan**

In 2002 the *Alberta Fusarium graminearum Management Plan* declared *F. gr.* a “regulated pest” and outlawed the importation or planting of cereal seed infected with *F. gr.* Coincidentally, the SIU began routine screening of Breeder seed for individual *Fusarium* species. Nine of the 29 *Fusarium*-positive Breeder seed lots produced at Indian Head in 2001 were found to be infected with *F. gr.* (Table 2).

It was immediately apparent that without an effective means of seed disinfection the SIU would be unable to meet the conditions of the Alberta *Fusarium graminearum* Management Plan. Breeder seed distribution to seed growers in Alberta was in jeopardy.

**A Solution at Hand?**

In early November 2001 Clear, Patrick, Turkington and Wallis (2001) presented a paper *Effect of Dry Heat Treatment on Fusarium graminearum* at the Canadian Workshop on Fusarium Head Blight (CWFHB). This paper described a method of dry heat disinfection versus seed borne *F. gr.*. The research showed that *Fusarium graminearum* was eliminated from wheat seed after 15 days at 60°C, 5 days at 70°C or 2 days at 80°C. *F. gr.* was eliminated from barley seed after 21 days at 60°C, 9 days at 70°C or 5 days at 80°C. Germination rates in wheat were relatively unaffected by the treatments while barley heated at 80°C showed a slight decrease in viability.

The Head of the SIU learned of the heat disinfection from the authors of this paper. By the end of November, 2001 SIU staff had treated samples from two Breeder seed lots with known FHB
infection. 1 kg samples of Superb hard red spring wheat and AC Ranger six row barley were disinfected.

The SIU’s method was similar to that of Clear et. al. with the exception that a **pretreatment phase** was added to minimize “cooking” of the seed. The treatment cycle had two distinct phases: 1) a 2 day pretreatment at 38°C and 2) a 5 day disinfection at 70°C. The pretreatment phase was designed to dry the bagged samples to <3% moisture content before raising the temperature to 70°C. 38°C is the maximum temperature recommended for drying commercial seed without damage to germination. The initial drying phase was added because moisture in the 1 kg bagged samples could not escape as readily as from the 200 seeds in open Petri plates used by Clear et. al. It was reasoned that higher moisture content in seed at the centre of the bags could cause seed damage by protein denaturation at 70°C = “cooking”.

Samples of both lots were taken: 1) before treatment, 2) after the initial 38°C drying phase and 3) after the 70°C disinfection phase. The samples were submitted to a local commercial seed lab where they were tested for germination and seed borne *Fusaria*. The results from these initial tests were very encouraging (Table 3).

**Dry Heat Disinfection of Larger Seed Lots**

Based on the results from the 1 kg wheat and barley samples, the SIU began a program of dry heat disinfection for cereal Breeder seed lots with known seed borne *Fusarium gr.*

Cereal Breeder seed is bagged in 15 kg units immediately after cleaning. To prevent contamination and for other practical reasons the seed was heat treated in the 15 kg bags. The facilities available for this were a large cabinet-type, forced-air sample dryer and a larger walk-in sample dryer. The smaller unit had electric heat with excellent controls and cross ventilation giving uniform temperature. However, its 400 kg batch capacity was insufficient to treat the amount of Breeder seed requiring disinfection. The larger shop-built, walk-in dryer was heated by a gas fired infra-red radiant emitter. It had a much higher batch capacity of 2.4 tonnes but poor air circulation in the chamber caused spatial temperature variability of 5°C when operated at 70°C. Both dryers were located in buildings >250 metres from the Seed Plant where Breeder seed was conditioned and stored so the seed had to be loaded onto a truck or trailer, hauled and manually unloaded and placed on racks or sample carts.

The larger walk-in dryer was chosen as the main heat treatment facility. To overcome the problem of uneven temperatures the carts on which the bagged seed was placed for treatment were manually rotated each day. To further compensate for uneven temperatures and the time taken up by daily movement of the seed the 38°C drying phase and the 70°C disinfection phase were lengthened by one and two days, respectively. Thus, the initial drying phase became 3 days followed by a 7 day disinfection phase for a total treatment cycle of 10 days.

During the winter of 2001/ 2002, 9 cereal Breeder lots (2398 kg) from the 2001 crop were disinfected in the walk-in sample dryer. These included 6 lots of spring wheat and 1 lot each of durum wheat, barley and oats. Testing by a commercial seed lab after disinfection confirmed that *F. gr.* was eradicated by the dry heat treatment (Table 4).
The following winter, 32 lots (16458 kg) of Breeder Seed produced in 2002 and 3 lots (1022 kg) produced in 2000 were disinfected in the walk-in dryer. Heat treatment eradicated seed borne *F. gr.* from all but one lot; a 178 kg lot of oat seed from the 2000 crop had 1% *F. gr.* after treatment (Table 4).

During the winter of 2003/ 2004, 16 lots (3814 kg) from the 2003 crop were treated. *F. gr.* was eradicated in all (Table 4).

The first three years of dry heat treatment proved the effectiveness of the method. *F. gr.* was eliminated from 59 Breeder seed lots with a combined total of 23514 kg. While this was a significant accomplishment it pushed both staff and equipment capacity to the limit. It was time for an upgrade.

In the spring of 2004 the SIU acquired a new walk-in oven for heat disinfection. The oven is located in the Seed Plant and is accessible to the unit’s forklift thereby greatly improving its ease of use. Its 3.6 tonne batch capacity is sufficient to keep pace with seed conditioning. Temperature control and cross ventilation within the chamber are excellent thus eliminating daily rotation of the seed and making it possible to reduce the disinfection phase by one day so that the treatment cycle could be shortened to 9 days. This oven enabled the SIU to incorporate heat disinfection as a routine procedure in its Breeder seed operation. Since purchase in 2004 the walk-in oven has been used to disinfect 60 seed lots with a combined total of 36407 kg. Its use has eliminated seed borne *F. gr.* from all seed lots excepting one 65 kg lot of barley in 2004 (Table 4). It has been a very valuable acquisition.

**Effect of Dry Heat Disinfection on Seed Viability**

To evaluate the effect of the dry heat treatment on seed viability, the initial germination of Breeder seed lots and their retention of viability over time were examined. Germination records of both treated and untreated lots were used.

The SIU is legally required to maintain current (annual) germination analyses for all Breeder seed lots on inventory. This provides a germination history of each lot from shortly after harvest until the lot is either expended or removed from inventory. Germination analyses were not routinely performed on untreated sub-samples from heat treated lots so direct comparison of germination histories of treated versus untreated seed from the same lots was not possible. However, it was possible to compare the initial germination and subsequent germination histories of treated versus untreated lots. For each crop kind arithmetic mean germinations were calculated based on years post-treatment or years post-harvest for treated and untreated lots, respectively (Tables 5, 6 & 7). Weather-damaged and sprouted lots were omitted because damaged seed is unsuitable for disinfection by dry heat, has substandard germination and loses viability rapidly under normal circumstances.

In most cases, germination testing indicated minimal effects of dry heat treatment on germination and retention of viability in ambient storage. After 3 years the mean germination of 5 disinfected wheat lots was only 0.4% lower than shortly after treatment (Table 5A). Similarly, after 3 years the mean germination of 10 untreated wheat lots was 0.4% lower than the initial post-harvest
Heat treatment did not damage the initial germination of wheat, barley or oats (Tables 5, 6 & 7). Viability of stored barley decreased more rapidly than that of wheat or oats for both treated and untreated lots (Tables 6A & 6B). Dry heat disinfection did not damage the viability or storage longevity of wheat, barley and oat seed lots with high initial germination. Dry heat treatment induced secondary dormancy in several seed lots. In these cases the germination 1 year post-treatment was >10% lower than the initial germination but recovered to near initial values by the third year (data not shown).

Acknowledgements
The diligent efforts, advice and support of R. Clear, S. Patrick, K. Turkington, R. Wallis, B. Kessel, W. Robb, S. Horsman, D. Reiss, J. Willoughby, M. Miller, J. Bole, R. Gehl and a host of seed analysts in commercial seed laboratories is gratefully acknowledged.

Reference

Table 1. Initial Fusarium* status of retained cereal Breeder seed lots from the AAFC Seed Increase Unit, Indian Head, Saskatchewan

<table>
<thead>
<tr>
<th>Year</th>
<th>Lots positive for Fusarium spp.*</th>
<th>Mean Infestation, %</th>
<th>Range of Infestation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>23: 23</td>
<td>5.1</td>
<td>1.0 - 13.5</td>
</tr>
<tr>
<td>2001</td>
<td>29: 32</td>
<td>1.2</td>
<td>0.0 - 7.0</td>
</tr>
<tr>
<td>2002</td>
<td>32: 32</td>
<td>3.3</td>
<td>0.5 - 12.0</td>
</tr>
<tr>
<td>2003</td>
<td>14: 34</td>
<td>1.1</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>2004</td>
<td>27: 30</td>
<td>2.0</td>
<td>0.0 - 5.5</td>
</tr>
<tr>
<td>2005</td>
<td>32: 32</td>
<td>3.0</td>
<td>0.5 - 9.0</td>
</tr>
<tr>
<td>2006</td>
<td>27: 27</td>
<td>5.5</td>
<td>0.5 - 20.0</td>
</tr>
<tr>
<td>sum</td>
<td>184: 210 (87.7%)</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

* Includes: F. graminearum, F. avenaceum, F. poae, F. culmorum, F. sporotrichioides, F. equiseti, F. acuminatum and F. proliferatum
Table 2. Initial *Fusarium graminearum* status of retained cereal Breeder seed lots from the AAFC Seed Increase Unit, Indian Head, Saskatchewan

<table>
<thead>
<tr>
<th>Year</th>
<th>Lots positive for <em>F. graminearum</em></th>
<th>Mean Infestation, %</th>
<th>Range of Infestation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>9: 32</td>
<td>1.1</td>
<td>0.0 - 3.5</td>
</tr>
<tr>
<td>2002</td>
<td>22: 32</td>
<td>1.1</td>
<td>0.0 - 3.0</td>
</tr>
<tr>
<td>2003</td>
<td>12: 34</td>
<td>1.0</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>2004</td>
<td>12: 30</td>
<td>1.1</td>
<td>0.0 - 2.5</td>
</tr>
<tr>
<td>2005</td>
<td>21: 32</td>
<td>1.2</td>
<td>0.0 - 4.0</td>
</tr>
<tr>
<td>2006</td>
<td>24: 27</td>
<td>3.8</td>
<td>0.5 -17.5</td>
</tr>
<tr>
<td>sum</td>
<td>100: 187 (53.5%)</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Effects of Dry Heat Treatment on 1kg Samples of Superb wheat and AC Ranger Barley, November 2001

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th><em>Fusarium spp.</em> %</th>
<th><em>Fusarium gr.</em> %</th>
<th>Germination %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC Ranger barley</td>
<td>None</td>
<td>0.5</td>
<td>0.5</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>2 days @ 38˚C</td>
<td>0.0</td>
<td>0.0</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>2 days @ 38˚C+ 5 days @ 70˚C</td>
<td>0.0</td>
<td>0.0</td>
<td>93</td>
</tr>
<tr>
<td>Superb wheat</td>
<td>None</td>
<td>4.5</td>
<td>0.0</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>2 days @ 38˚C</td>
<td>4.0</td>
<td>0.0</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>2 days @ 38˚C+ 5 days @ 70˚C</td>
<td>0.0</td>
<td>0.0</td>
<td>96</td>
</tr>
</tbody>
</table>
Table 4. Fusarium status of cereal Breeder seed lots after dry heat disinfection

<table>
<thead>
<tr>
<th>Year</th>
<th>Lots disinfected # (kg)</th>
<th>Lots positive for <em>F. spp.</em> after treatment</th>
<th>Lots positive for <em>F. gr.</em> after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>3* (1022)</td>
<td>1</td>
<td>1**</td>
</tr>
<tr>
<td>2001</td>
<td>9 (2398)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2002</td>
<td>32 (16458)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>16 (3814)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>12 (3116)</td>
<td>12</td>
<td>1***</td>
</tr>
<tr>
<td>2005</td>
<td>32 (20871)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>27 (12420)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>sum</td>
<td>131 (60099)</td>
<td>35: 88</td>
<td>2:131</td>
</tr>
</tbody>
</table>

* 3 lots from the 2000 harvest were disinfected during the winter of 2002 / 2003.
** 1 - 178 kg lot of standard oats tested 1.0% *F. gr.* after treatment; 2.5% before.
*** 1 - 65 kg lot of six row barley tested 0.5% *F. gr.* after treatment; 0.5% before.

Table 5. Germination Histories of Wheat Breeder Seed Lots

A. Mean Germination of Heat Treated Lots, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.4 (43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96.1 (28)</td>
<td>95.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95.9 (14)</td>
<td>96.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96.4 (5)</td>
<td>96.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.0 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90.0</td>
</tr>
</tbody>
</table>

B. Mean Germination of Untreated Seed Lots, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-harvest</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.3 (19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94.3 (17)</td>
<td>94.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.2 (11)</td>
<td>93.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.1 (10)</td>
<td></td>
<td></td>
<td></td>
<td>92.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.4 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95.6</td>
<td></td>
</tr>
<tr>
<td>93.4 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91.2</td>
</tr>
</tbody>
</table>
Table 6. Germination Histories of Barley Breeder Seed Lots

A. Mean Germination of Heat Treated Lots, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.9 (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.25 (12)</td>
<td>89.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.8 (5)</td>
<td></td>
<td>94.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5 (2)</td>
<td></td>
<td></td>
<td>89.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.0 (1)</td>
<td></td>
<td></td>
<td></td>
<td>83.0</td>
</tr>
</tbody>
</table>

B. Mean Germination of Untreated Seed Lots, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-harvest</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97.0 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.6 (5)</td>
<td>95.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.6 (5)</td>
<td></td>
<td>90.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.0 (2)</td>
<td></td>
<td></td>
<td>81.5</td>
</tr>
</tbody>
</table>

Table 7. Germination Histories of Oat Breeder Seed Lots

A. Mean Germination of Heat Treated Lots*, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96.3 (14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.1 (11)</td>
<td>93.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95.5 (6)</td>
<td></td>
<td>96.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95.4 (5)</td>
<td></td>
<td></td>
<td>94.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.0 (1)</td>
<td></td>
<td></td>
<td></td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.0 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95.0</td>
</tr>
</tbody>
</table>

B. Mean Germination of Untreated Lots*, % (# lots used to calculate means)

<table>
<thead>
<tr>
<th>Years Post-harvest</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.4 (16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.3 (13)</td>
<td>94.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95.4 (9)</td>
<td></td>
<td>92.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.0 (11)</td>
<td></td>
<td></td>
<td>95.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.6 (5)</td>
<td></td>
<td></td>
<td></td>
<td>79.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.6 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87.2</td>
</tr>
</tbody>
</table>

* Does not include hulless oat varieties.
FHB Epidemic risk forecasting system: A Northern Great Plains perspective.
Charla R. Hollingsworth and Marcia McMullen University of Minnesota Northwest Research and Outreach Center and Department of Plant Pathology, 2900 University Avenue, Crookston, MN 56716 U.S.A; Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105 U.S.A.

During the 1990s, a cooperative multi-state effort among researchers at land grant universities produced the first iteration of epidemic risk models designed to predict Fusarium head blight (FHB) epidemics and non-epidemics. This expanding group of cooperators annually share weather data (hourly air temperature, dew point, and rainfall), crop growth stage information, and FHB disease responses on wheat and barley from multi-site, replicated experiments to support the development of epidemic risk forecasting models with increasingly accurate predictive abilities.

Until recently, the Minnesota small grain crops production community relied exclusively on traditional tools to manage losses caused by FHB. Many producers understand that management of FHB includes an integrated approach such as growing cultivars with resistance, applying fungicide at the early flowering growth stage (Feekes 10.51), and managing infested crop residue with rotation and/or tillage. Epidemic risk predictions are used as decision-making tools prior to the time when an application of fungicide should be made to manage disease. A statewide FHB epidemic risk forecasting system has been operational for producers in Minnesota at http://mawg.cropdisease.com since 2004, and for producers in North Dakota at http://www.ag.ndsu.nodak.edu/cropdisease since 2000. Agricultural professionals, producers, and others can access either system free of charge to assist in determining whether the risk of crop loss from disease is likely and would warrant incurring additional input costs associated with fungicide application.

Operation and maintenance of the Minnesota FHB epidemic risk forecasting system website is funded by the Minnesota Wheat Research and Promotion Council. The site is maintained by a private company, Meridian Environmental Technology, Inc. (Meridian) in cooperation with the University of Minnesota. This Grand Forks, North Dakota, U.S.A. company maintains a comprehensive weather database that integrates data collected from surface-observed stations and remotely-sensed information from weather radars and satellites. Observed weather throughout Minnesota is recorded by federal and state agencies such as the National Weather Service and the Federal Aviation Administration (>82 stations), the Minnesota Department of Transportation, Road Weather Information System (93 stations), and the North Dakota Agricultural Weather Network (NDAWN; 10 stations). Remotely-sensed information originates from six NEXt Generation Weather RADar (NEXRAD) weather radars. The outcome is a 4-km resolution composite of hourly precipitation, temperature, and humidity data which is used to update the forecasting system every three hours, for a total of eight times a day.

The North Dakota FHB epidemic risk forecasting system website began in 2000, with forecasting provided through 24 NDAWN locations. Since that time, the NDAWN system, and consequently the epidemic risk forecasting system, has expanded to 70 locations, 60 in North Dakota and 10 in Minnesota. The North Dakota forecasting
website averages between 7000 and 8000 visits from unique web addresses each year, with the peak accesses in late June and early July.

The epidemic forecasting system model equation and its risk value thresholds are modified yearly or as needed by a core group of researchers. The U.S. Wheat and Barley Scab Initiative has supported model development and modification efforts for a number of years by funding scientists at Kansas State University, Penn State University, The Ohio State University, and North Dakota State University, among others. Since 2004, a number of key modifications have been made. Categorical designations for calculated risk values, known as ‘thresholds for application’, are frequently modified (Table 1). The trend toward increasing the upper limit threshold of the low epidemic risk designation and increasing the lower limit threshold of the high epidemic risk designation reduces the number of false-positive epidemic predictions made by the model. Predicting epidemics when none are observed has occurred during two of four years the system has been ground-truthed in Minnesota.

<table>
<thead>
<tr>
<th>Year</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>≤40</td>
<td>41–47</td>
<td>≥48</td>
</tr>
<tr>
<td>2005</td>
<td>≤46</td>
<td>47–63</td>
<td>≥64</td>
</tr>
<tr>
<td>2006</td>
<td>≤50</td>
<td>51–79</td>
<td>≥80</td>
</tr>
<tr>
<td>2007</td>
<td>≤50</td>
<td>51–79</td>
<td>≥80</td>
</tr>
</tbody>
</table>

During 2004, five on-farm disease management experiments were conducted within commercial production fields located from north to south in the Red River Valley (near Kittson, Strathcona, Oklee, Perley, and Fergus Falls). During 2005, experiments were again planted at similar locations (near Kittson, Strathcona, Oklee, Perley, and Rothsay). Unfortunately, stand losses due to severe weather and flooding resulted in tests at the Kittson and Strathcona locations being discontinued. During 2006 and 2007, tests were again planted near Oklee and Fergus Falls. In total, from 2004 to 2007, 19 environments (location + cultivar flowering period) with ground-truthing data were collected from replicated, small-plot experiments consisting of data from five to 13 cultivars. Data from non-fungicide treated control plots were used to support the ground-truthing effort.

During 2004, the risk forecasting model predicted an elevated (moderate to high) risk that an FHB epidemic would occur in two of five (40%) environments, and a low risk that an epidemic would occur in three of five (60%) environments. No epidemics were observed. Single year model prediction accuracy was 60% for non-epidemics and 0% for epidemics. During 2005, it predicted an elevated risk that an epidemic would occur in one of six (17%) environments, and a low risk that an epidemic would occur in five of six (83%) environments. Two epidemics were observed. Single year model prediction accuracy was 80% for non-epidemics and 50% for epidemics. During 2006, the model predicted a low risk that an epidemic would occur in four of four environments (100%), and no epidemics were observed. Single year model prediction accuracy was 100% for
non-epidemics. Again during 2007, the model predicted a low risk that an epidemic would occur in four of four environments (100%), and no epidemics were observed. Single year model prediction accuracy was 100% for non-epidemics. From 2004 to 2007, non-epidemics were predicted accurately 94% of the time and epidemics were predicted accurately 66% of the time. The model under-predicted non-epidemics in 6% of the environments and over-predicted epidemics in 33% of the environments. Model modifications made after the 2005 growing season meant to increase the accuracy for predicting epidemics have not yet been challenged in Minnesota because no epidemics have occurred since then.

Since 2006, the spring wheat epidemic risk forecasting model has included an additional equation term representing FHB resistance levels of cultivars. Users have the option of selecting either a specific cultivar of interest, or a generic FHB resistance level (very susceptible, susceptible, moderately susceptible, moderately resistant). This model modification allows for a more precise prediction in that specific variables such as location-specific weather data and cultivar response to FHB are known. It is likely that incorporating FHB resistance levels of cultivars into the model may be one of the most beneficial modifications made in recent years.

Deployed since 2004, the national FHB epidemic risk forecasting system website located at [http://www.wheatscab.psu.edu](http://www.wheatscab.psu.edu) is supported by the U.S. Wheat and Barley Scab Initiative. Epidemic risk forecasts are available free of charge. The system services the central and eastern U.S. wheat community located in 24 states, including Minnesota and North Dakota. A number of weather data sources are used by the national model system, with the most substantial contributions provided by federal agencies. The national epidemic risk forecasting system relies primarily on remote-sensed weather data devices, whereas the Minnesota and North Dakota systems rely primarily on observed station data. A disease forecasting component based on 24 hour and 48 hour weather forecasts was added to the national model in during 2006. Minnesota and North Dakota hope to incorporate weather forecasts into their FHB epidemic risk forecasting websites, as well.

**Integrated Strategies for FHB Management, a Northern Great Plains Perspective.** Marcia McMullen. *Dept. of Plant Pathology, North Dakota State University, Fargo, ND, 58105, USA*

Environmental conditions affect the development of Fusarium head blight (FHB) and associated mycotoxins. Few would argue that climatic conditions affect all stages of the life cycle of *Fusarium graminearum*, and favorable climate during vulnerable crop growth stages often is the key factor resulting in severe disease (Andersen, 1948; Champeil, et al., 2004; Nakagawa, et al., 1966; Parry, et al., 1995; Snidjers, 1990; Sutton, 1982).

As weather conditions are often difficult to predict long-term, and difficult to avoid, researchers and producers have looked for implementable strategies for managing FHB.
Champeil, et al., 2004, provided an extensive review of cultural practices that have been studied that may affect FHB severity and mycotoxin production. Although not the only strategies discussed, some key strategies extensively researched include: crop rotation, residue management, tolerant cultivars, and fungicide use (Bai and Shaner, 1994; Champeil, et al., 2003; Dill-Macky and Jones, 2000; Mesterhazy, 2003; Obst, et al. 1999; Pageau, et al., 2005; Paul, et al., 2007; Rioux, 2005; Salas and Dill-Macky, 2005; Sutton, 1982; and Vogelgsang and Forrer, 2005). These studies have shown success with one or more strategies in reducing FHB and/or mycotoxins, but few have shown the quantitative effect of combining multiple strategies (Champeil, et al. 2004).

From 2003-2005, various regions in the US had FHB outbreaks, and individual FHB management strategies did not necessarily reduce FHB severity and deoxynivalenol (DON) to levels required by the grain industry. In 2005, parts of eastern North Dakota (ND) experienced favorable environmental conditions during wheat anthesis and a subsequent FHB outbreak occurred. Several research trials in eastern ND that year gave supportive, quantitative evidence that a combination of practices - crop rotation, variety choice, and fungicide use - reduced FHB severity and DON levels in an additive manner (Table 1).

Table 1. Influence of management practices on Fusarium head blight (FHB) and deoxynivalenol (DON), across two eastern North Dakota State University research locations, Fargo and Prosper, 2005

<table>
<thead>
<tr>
<th>FHB Management Practice</th>
<th>Total FHB reduction %</th>
<th>Actual FHB Severity Index1 %</th>
<th>Total DON reduction %</th>
<th>Actual DON ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Rotation alone: soybean residue instead of wheat</td>
<td>50</td>
<td>20</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Rotation + Variety: Alsen - MR rx instead of Reeder - S rx</td>
<td>80</td>
<td>8</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Rotation + Variety + Fungicide: Folicur fungicide (4 fl oz/A + 0.125% v/v Induce) at flowering</td>
<td>92</td>
<td>3.2</td>
<td>88</td>
<td>1.2</td>
</tr>
</tbody>
</table>

1 FHB severity index = (incidence of tillers with symptoms x head severity on infected tillers)/100

These results were part of the discussion when members of the management group of the US Wheat and Barley Scab Initiative (USWBSI) met in Feb., 2006 and decided to develop studies across states and grain classes aimed at quantifying the value of additive strategies for FHB and DON management. These studies would be an expansion of the epidemiology cooperative study that was ongoing in the initiative, a study which generally used artificial levels or sources of inoculum, but which had indicated that combinations of two or more management practices resulted in better FHB control (Nita, et al., 2005).

The new cropping systems studies were to be done under natural field conditions and the objectives were to:
1) demonstrate that integrated management is the most effective means of reducing losses to FHB/DON; and

2) increase grower adoption of integrated strategies by demonstration of their effectiveness in a wide range of environments.

Proposals to the USWBSI to do collaborative cropping system studies were approved in 2006, with studies in place in 2007, a year in which some locations again had FHB. Examples of results from North Dakota follow: additional information on studies from other parts of the US will be presented at the US FHB Forum in Kansas City in December.

In ND in 2007, some research trials examined the additive effect of resistance and fungicides and others examined the additive effects of rotation, cultivar and fungicide. Moderate to high levels of FHB occurred in the winter wheat trial example (Table 2), moderate levels of FHB occurred in the durum trial example (Table 3), and very low FHB levels occurred in the spring wheat trial example (Table 4). Additive effects of management practices were most evident in the winter wheat and durum trials.

**Table 2.** Winter wheat cropping system study, Lisbon, ND, 2007 (Joel Ransom, Marcia McMullen, Scott Meyer) (Over all 20 varieties included in study: variety by fungicide interactions significant for: yield*, test weight**, FHB field severity**, DON at P=0.08. The following table compares results from two of the most resistant lines[‘Jerry’ and ‘Millennium’] vs two of most susceptible [‘CDC Falcon’ and ‘Jagalene’])

<table>
<thead>
<tr>
<th>FHB Management Practice</th>
<th>Total FHB reduction %</th>
<th>Actual FHB Severity Index %</th>
<th>Total DON reduction %</th>
<th>Actual DONd ppm</th>
<th>Yield bu/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (average of susceptible(^a), no fungicide)</td>
<td>0</td>
<td>26.8</td>
<td>0</td>
<td>1.7</td>
<td>50.6</td>
</tr>
<tr>
<td>+ Variety resistance(^b) (or + Fungicide only)</td>
<td>86 (82.7)</td>
<td>3.7 (5)</td>
<td>64.7 (75.4)</td>
<td>0.6 (0.5)</td>
<td>69.2 (76.3)</td>
</tr>
<tr>
<td>Fungicide(^c) + Variety resistance</td>
<td>98</td>
<td>0.5</td>
<td>82.4</td>
<td>0.3</td>
<td>86.1</td>
</tr>
</tbody>
</table>

\(^a\) two susceptible lines averaged (‘CDC Falcon’ and ‘Jagalene’)

\(^b\) two resistant lines averaged (‘Jerry’ and ‘Millennium’)

\(^c\) Proline + Folicur (3 fl oz/A each + 0.125% v/v Induce) applied at flowering stage of each variety

\(^d\) DON values relatively low, perhaps because environment turned hot and dry after soft dough stage and infected kernels may have shriveled and not been included in harvested sample.
Table 3. Durum Cropping System Study, Langdon Research Extension Center, ND 2007
(study coordinated by Scott Halley)

<table>
<thead>
<tr>
<th>Previous Crop</th>
<th>Fungicide Treatment</th>
<th>FHB Incid. %</th>
<th>FHB Index %</th>
<th>DON ppm</th>
<th>Leaf spot %</th>
<th>Yield bu/a</th>
<th>Twt lbs/bu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola</td>
<td>Yes</td>
<td>54.9</td>
<td>6.2</td>
<td>0.88</td>
<td>72.3</td>
<td>70.0</td>
<td>60.7</td>
</tr>
<tr>
<td>Canola</td>
<td>No</td>
<td>82.4</td>
<td>19.1</td>
<td>2.06</td>
<td>78.5</td>
<td>62.1</td>
<td>59.8</td>
</tr>
<tr>
<td>HRSW</td>
<td>Yes</td>
<td>68.0</td>
<td>9.1</td>
<td>1.91</td>
<td>49.9</td>
<td>57.0</td>
<td>58.4</td>
</tr>
<tr>
<td>HRSW</td>
<td>No</td>
<td>88.3</td>
<td>21.0</td>
<td>2.78</td>
<td>53.3</td>
<td>51.1</td>
<td>57.9</td>
</tr>
</tbody>
</table>

Across all varieties

<table>
<thead>
<tr>
<th>Avg. Across Var. + crop</th>
<th>*</th>
<th>NS</th>
<th>*</th>
<th>*</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>*</th>
<th>*</th>
</tr>
</thead>
</table>

Avg. Across

| Canola | 68.6 | 12.6 | 1.59 | 75.4 | 66.1 | 60.2 |
| HRSW  | 77.6 | 15.0 | 2.34 | 55.3 | 54.0 | 58.1 |

Highlighted #s are highest average FHB index, average lowest yield, with wheat as previous crop and no fungicide treatment.

‘Divide’ (MR) averaged the lowest FHB index (9.9) and ‘Monroe’ (S) the highest FHB index (19.1). ‘Grenora’ had the lowest DON (0.97 ppm), on canola ground and treated with fungicide. ‘Grenora’ (MR) had the highest yield, on canola ground + fungicide = 71.9 bu/a; ‘Monroe’ (S) had lowest yield, on HRSW ground and no fungicide = 48.7 bu/a.

Table 4. Hard red spring wheat (HRSW) cropping system study, Fargo, ND 2007
(Marcia McMullen, Jim Jordahl, Scott Meyer)

<table>
<thead>
<tr>
<th>Previous Crop</th>
<th>Fungicide Treatment</th>
<th>FHB Incid. %</th>
<th>FHB Index %</th>
<th>Leaf rust %</th>
<th>Leaf spot %</th>
<th>Yield bu/a</th>
<th>Twt lbs/bu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>Yes</td>
<td>10.0</td>
<td>0.6</td>
<td>0.1</td>
<td>0.7</td>
<td>47.2</td>
<td>57.7</td>
</tr>
<tr>
<td>Soybean</td>
<td>No</td>
<td>21.3</td>
<td>1.2</td>
<td>8.7</td>
<td>1.7</td>
<td>43.7</td>
<td>57.5</td>
</tr>
<tr>
<td>HRSW</td>
<td>Yes</td>
<td>10.9</td>
<td>0.4</td>
<td>0.0</td>
<td>0.6</td>
<td>45.5</td>
<td>58.8</td>
</tr>
<tr>
<td>HRSW</td>
<td>No</td>
<td>21.9</td>
<td>1.5</td>
<td>1.8</td>
<td>1.6</td>
<td>42.2</td>
<td>58.4</td>
</tr>
</tbody>
</table>

Across all varieties

<table>
<thead>
<tr>
<th>Avg. Across Var. + crop</th>
<th>*</th>
<th>*</th>
<th>NS</th>
<th>*</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>*</th>
<th>*</th>
</tr>
</thead>
</table>

Avg. Across

<table>
<thead>
<tr>
<th>Fungicide Treatment</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
<th>*</th>
</tr>
</thead>
</table>

Avg. Across

| Soybean | 15.6 | 0.9 | 2.2 | 1.2 | 45.4 | 57.6 |
| HRSW    | 16.9 | 0.9 | 0.9 | 1.1 | 43.9 | 58.5 |

Highlighted #s are highest average FHB index and average lowest yield, with wheat as previous crop and no fungicide treatment.
'Briggs’ spring wheat (susceptible) surpassed the FHB resistant ‘Glenn’ in yield (by 4 bushels over all treatments) because it is more tolerant of heat stress, but ‘Glenn’ had significantly lowest FHB Index (0.2). Susceptibility to both Fusarium head blight and leaf rust contributed to ‘Trooper’ having the lowest yield (40.9 bu/a) and test weight (55.9 lbs/bu) across all treatments. DON levels were all below 0.5 ppm because of low FHB.

**Literature Cited:**


93
Fusarium head blight (FHB) (as caused by Fusarium graminearum Schwabe) has been an important disease problem in cereals in the Atlantic Region of Canada, since the early 1980's. Over the last four years the severity has been high in one or more production areas in the region; with mycotoxin (deoxynivalenol, DON) levels sometimes in excess of 10ppm. The Region can be classified as a high moisture environment, which presents a challenge in providing for adequate disease reduction strategies for field symptoms and mycotoxin contamination, and in understanding the dynamics of epidemics. The infection period extends well beyond anthesis and multiple infection periods do occur, as is evident from infection curves. This presents a challenge in achieving fungicidal control, which has been variable between years, usually giving a maximum control of only about 50%. Rotations used in the region would normally be classified as being favorable for minimizing FHB; with a low incidence of corn production and extensive use of non-host crops between the cereal crops. However the severity of epidemics would indicate that this is not sufficient to provide for adequate disease reduction. While not in itself a control mechanism for FHB development, dehulling of barley has proven, in preliminary studies in the Region, to be very effective at significantly reducing DON levels. Ultimately, in this environment, the use of low risk cereal species or resistance would appear to be the best option for FHB management.
Introduction

Fusarium Head Blight (FHB) is a disease caused by several species of *Fusarium*. In Argentina, 90% of the pathogens isolated from blighted heads were *Fusarium graminearum* Schwabe [teleomorph Gibberella zeae Schwein (Petch). The Argentinean wheat cropping area is very extensive (nearly 6,000,000 ha), distributed in five provinces with different ecological conditions (Pampas region: Buenos Aires, Córdoba, Santa Fe, Entre Ríos and La Pampa). Therefore, no one epidemic has ever covered the whole area at a given time. Latitude, temperature and the importance of susceptible grass weeds and rotational crops (particularly *Zea mays* L.) influence the pathogen’s distribution, while the frequency and timing of spring rainfall appear to regulate disease outbreaks.


Meteorological-based FHB incidence predictive models. Empirical approach

A- Model Development

Applying linear regression techniques, different models up to a maximum of three independent meteorological variables were fitted to FHB incidence data, in Pergamino (33°56’S, 60°30’W, humid Pampas region) (Moschini and Fortugno, 1996). Annual mean percent disease incidence (n=22) computed from the values recorded for the wheat cultivars grouped by their similar heading dates were used to fit the models. Using daily records of maximum and minimum temperature, precipitation and relative humidity (average of the 0800, 1400 and 2000 h observations) obtained from a standard weather station, independent meteorological variables were calculated. The closest associations between environmental variables and FHB incidence data were obtained in a time period beginning eight days prior to heading date (when 50% of the heads were fully emerged: emergence of first heads) and ending when 530 degree days (DD) were accumulated (base temperature: 0° C). This period was regarded as the critical period for infection (CPI) lasted 26 to 32 days in this study.

The number of two-day periods with precipitation (≥0.2mm) and relative humidity > 81% in the first day and relative humidity ≥ 78% in the second day (*NP*) was the variable that showed the strongest association with disease incidence (R²: 0.81). Long wet-anther periods (48-60 h) favor the infection of the pathogen. Not having measurements of duration of rainfall wetness events, their potential lengths was better considered by combining the occurrence of precipitation with high air relative humidity records in a two-day window. A low correlation was found between precipitation
frequency and disease incidence ($R^2=0.17$). The equation [1] was finally selected for predicting FHB incidence ($PFHBI\%$)

$$PFHBI\%=20.37 + 8.63 \times NP - 0.49 \times DD$$

$R^2=0.86$

[1] in which $DD$ represents the daily accumulation of the residuals resulting from subtracting 9 to the minimum temperature values (<9°C) and the exceeding amounts of maximum temperatures from 26 °C. This equation was adjusted and validated for northern and southern locations than Pergamino (Moschini et al., 2001; Moschini et al., 2004; Carranza et al., 2007)

**B- Model Applications (Eq 1 and adjustments)**

- Climate risk of the Pampas region regarding the FHB incidence

![Figure 1](image)

Figure 1: Percentage of years with occurrence of severe FHB epidemics

For 36 stations of the Pampas region with daily meteorological data for the period 1971-2006, levels of FHB incidence for each year were estimated by the Eq. 1 and adjustments. The probability of occurrence of severe FHB incidence (considered here as >45 %) was calculated for each station. The spatial distribution of these probabilities for the Pampas region can be observed in Figure 1. North-eastern Pampas region area showed the highest probability of severe attacks (4 to 6 severe epidemics out of 20 years). The climate risk of the region regarding the disease gradually decreases towards the south-west.

- Early knowledge (before wheat harvest) of the spatial distribution of predicted FHB incidence values
This work was done for satisfying mill requirements. Per wheat growing season and before harvest, for the 36 stations of the Pampas region, levels of FHB incidence were estimated by Eq. 1 and adjustments. The spatial distribution of these predicted FHB values can be observed in Figure 2 for the contrasted 2001 and 2006 wheat growing season.

- Impact of phenomena that produce inter annual climate variability (El Niño-Southern Oscillation: ENSO) regarding FHB incidence

On inter annual time scale, El Niño Southern Oscillation (ENSO) is the most important coupled ocean-atmosphere phenomenon to cause global climate variability. ENSO recognizes one neutral and two extreme phases: El Niño and La Niña. Annual deviations between predicted FHB incidence values (Eq. 1) and the mean were graphically analyzed in relation to ENSO phases in Parana (31°50'S, 60°31’W) and Balcarce (37°45’S, 58°18’W) (period 1971-2006) (Figure 3). In Paraná, 7 out of 11 cold events were below the mean. An erratic trend was observed for warm El Niño event. Severe observed FHB epidemics (1978, 1985, 1993 and 2001) resulted to be neutral. In contrast, Balcarce showed a stronger ENSO related positive disease deviations from the mean for El Niño episodes (9 out of 12) and negative for La Niña (9 out of 11). Many references pointed out the occurrence of more frequent rainfalls since November in El Niño years. The delay of wheat heading date in the south helps to overlay the critical period for the infection with the rainfall period. The more homogenous disease response to La Niña episodes in the two locations is in agreement with the extended occurrence of lesser than normal probability of precipitation in La Niña years.
Fusarium index predictive system. Fundamental-empirical approach

A- Development (Moschini et al., 2002; validations: Moschini et al., 2004; Carranza et al., 2007): a total of 84 Fusarium index values (incidence% x severity% / 100) registered in commercial wheat cultivars (susceptible and moderately susceptible) at Pergamino and M. Juárez (32°41’S, 62°07’W). (10 crop seasons) were satisfactorily contrasted with predicted values using a fundamental-empirical approach.

Predicted Fusarium index values were obtained following the next steps:

a) Daily progress of anthesis (% of wheat heads with exposed anthers): from field observations in a single wheat cultivar, a polynomial function between the logit of the proportion of head with anthers (Anther, values from 0 to 1) and the time in degree days (DD: daily accumulation of mean temperatures >= to 12 °C) was fit.

\[
\text{LogitAnther} = -6.76502912 + 0.136395967 \times \text{DD} - 0.000694621 \times \text{DD}^2 + 0.000001384 \times \text{DD}^3 - 0.000000001 \times \text{DD}^4
\]

[2]
where LogitAnther is the natural logarithm of (Anther / 1- Anther), DD²=DD x DD; DD³=DD² x DD, DD⁴= DD x DD³. Solving (EXP(LogitAnther) / (1+EXP(LogitAnther))) x 100, the daily percentage of heads with anthers were obtained. The CPI began 4 days prior the observed heading date and finished when 530 DD were accumulated.

b) Predicted severity: in controlled environment, Andersen (1948) established the percentage of infection (severity%) in wheat heads inoculated with *Fusarium graminearum* conidia, exposed to different wetness periods (W: from 18 h to 72 h) and temperatures (T=15, 20, 25 and 30 °C). A polynomial function was fit between the logit of the severity (S, from:0 to 1) and W (h) and T (°C), like individual and interactive effects.

$$\text{LogitS} = 38.77166158 - 0.53815698 \times W - 6.02985565 \times T + 0.26849793 \times T^2 - 0.00396097 \times T^3 + 0.04990941 \times IT - 0.00092343 \times IT^2$$  \[3\]

where LogitS is the natural logarithm of (S / 1-S); T²=T x T; T³=T² x T; IT=T x W; IT²=T² x W. Values of predicted severity were obtained solving EXP(LogitS)/(1+EXP(LogitS))x 100.

In order to use Eq. [3], equivalence rules defining W values and mean T during wet periods from daily precipitation (Prec), maximum and minimum temperature and relative humidity (RH%) registered at standard weather stations, were established. Using criteria derived from empirical approach (Moschini and Fortugno 1996), it was defined that:

- 1 day with Prec (>=0,2mm) and RH>= 81% is equal to W=24 h.
- 2 consecutive days with Prec and RH >= 81% is equal to W=48 h.
- 3 consecutive days with Prec and RH >=81% is equal to W=72 h. A maximum W period of 72 h was analyzed. If prior and/or after W period of 24 and 48 h, Prec and RH<=77 % are registered, 3 h of wet are added. If Prec and RH >77% and <81% (prior and/or after) are registered, 6 h of wet are added. Occurrence of RH>77 % and <81% after W periods, 3 h are added. The temperature during the wet period resulted of averaging the mean daily temperatures (T= \( \frac{\text{MaxT+MinT}}{2} \)), weighted for the wet duration in each day involved. If T is <15°C, severity values are only calculated for wet periods >= 48 h.

c) The final predicted Fusarium index value resulted of adding the partial products between a) and b) (divided by 100), calculated for all the wet periods found throughout the wheat CPI.

**B- Application.** Synoptic weather patterns related to FHB infection

FHB infective event severities were estimated (Eq. 3) in 5 sites (eastern Pampas region) throughout 45-day periods (in which wheat head with anthers could be available) for the growing seasons 1971-2006. FHB severities (n=731) were categorized into levels: severe (S>3.7 %, percentile 75%), moderate (S<=3.7% and >1%), light (S<=1%, percentile 25%). In Paraná, 51 and 27 FHB infective events were identified as severe and light respectively, including their initial days. From NCEP/NCAR reanalysis (Kalnay et al., 1996), three-day sequences (initial day and the 2 previous) of mean daily 1000 Hpa geopotential height maps (grid resolution: 2.5°x 2.5°) were obtained for south America (130° to 40° W; 20° to 60°S). Using the technique developed by Lund (1963), each 3-day sequence of mean daily 1000 Hpa geopotential height (gh) map was correlate (r: Pearson correlation coefficient) with all the other 3-day sequences analyzed. In Paraná, one 3-day
sequence map (initial day infection event: 12 October 1993) had the most $r \geq 0.70$ (33 out of 51). This recurring map-pattern configuration was designated as Type As. The same process was carried out for 27 light infection events. In this case, one 3-day sequence map (initial day: 16 September 1982) had the most $r \geq 0.70$ (14 out of 27) (Type Al). Figure 4 shows both type A synoptic patterns, resulting of averaging (point by point of the 1000 Hpa gh grid) the 33 (severe infections) and 14 (light infections) maps corresponding to the initial day of FHB infection event.

![Synoptic weather patterns (1000 HPa geopotential height) related to severe (Type As, upper) and light (Type Al, bottom) FHB infection events (initial day of FHB infection)](image)

Type As synoptic pattern related to severe FHB events presented a low pressure centre located central-north of Argentina, involving ascendant air inducing precipitations (primary source for wetting periods required for FHB infections). When the three-day sequence (initial day and the 2 previous) of 1000 Hpa gh was analyzed, two strong anticyclones (Pacific and Atlantic) and a low pressure axis over Argentina was characteristic. The strong Atlantic high pressure centre produced advection of warm and humid air during the two previous days to the beginning of FHB infection episodes.

Type Al synoptic pattern related to light FHB events showed a latitudinal high pressure axis and two low pressure areas, one strong over central northern Argentina and the other southern. The weak Atlantic high pressure centre produced the incoming of
northern air mass with less humidity comparing to Type As. The strong Pacific anticyclone circulation pushed rapidly the continental cyclone to the north of Argentina. This fast process might explain the occurrence of short wetting period (originated by precipitation) related to light FHB infection events.

**Operational system for assessing FHB risk**

Multiple inoculation episodes in areas with moderate and severe outbreaks suggest that multiple infections contribute to cumulative head blight severity. This fact affects prophylactic disease management options (Francl et al. 1999). Keeping the latter in mind, conclusions derived from the fundamental-empirical system can be used to assist producers in disease control measures to be employed. From the start of the wheat critical period for infection (onset of wheat heading), environment monitoring can detect infection events and the corresponding predicted Fusarium index values, in order to decide a possible crop chemical protection. Regarding the meteorological-based empirical equations, it should be underlined the fact that their meteorological variables are calculated after finishing the entire wheat critical period for infection. At this point, the impact of having the estimated disease incidence from the equations could be not useful for the decision-making process respecting a chemical control. Nevertheless, the identification of the meteorological variables highly associated with the disease might be helpful to analyze weather forecasts. Accordingly, the probability of occurrence of 2 to 3 days periods of rainfall and high humidity and above normal temperatures derived from weather forecasts should be evaluated throughout the wheat CPI.

Since 2005/06 wheat growing season, a system for assessing FHB risk was implemented for the Pampas region (divided in three sub-region: North, Central and South). The system, three times a week (Monday, Wednesday and Friday), updates daily meteorological data from 45 standard weather stations for running predictive FHB models (empirical and fundamental-empirical approaches). Also, an specific weather forecast is elaborated. After analyzing all together (forecast and models), comments and maps showing the spatial distribution of three possible FHB risk grade: high (red), moderate (yellow) and/or low (green), are presented at the web page: [www.intacya.org](http://www.intacya.org) (Instituto de Clima y Agua).

**References**


Quality and End-Use Safety/ Qualité et sécurité

The Malting & Brewing Perspective on FHB in Malting Barley. E. Armstrong. Brewing and Malting Barley Research Institute, 303 – 161 Portage Avenue East, Winnipeg MB R3B 2L6, Canada.

The Brewing and Malting Barley Research Institute (BMBRI) maintains a list of ‘Desirable Quality Traits in Malting Barley’ and makes this list available directly to barley breeders and researchers, as well as to any other interested parties through its web site (www.bmbri.ca). ‘FHB resistance’ has been on the list for many years and remains an important breeding target. As a plant disease resistance target, it is one of the few items on the list that is not specifically related to a processing quality trait. However, FHB resistance, and more specifically DON content of malting barley, are critically important to the quality of malt and beer which can be produced. DON levels are associated with ‘gushing’, an unacceptable quality trait in the finished product. Because of this association, there are strict limits to the levels of DON that will be accepted, in many cases zero, during barley selection for malting. This of course has an impact on the overall selection rates of malting barley from areas which are affected by Fusarium head blight, and therefore also has an impact on the amount of barley grown in these areas, limiting production in the Red River Valley in particular. The presentation will review the quality impact of DON on malting and brewing, as well as the impact of FHB incidence on barley availability for the industry.

Infections of cereal grains by *fusarium* not only lead to reductions in crop yields and quality, but also lead to a reduction in the suitability of the grain as a feed source for livestock. The latter is due to the fact that mycotoxins, most notably deoxynivalenol (DON), produced by the fungi can suppress feed intake, with swine being particularly sensitive. Current guidelines recommend keeping DON levels to less than 1 ppm in the final ration for growing swine, and diets for pregnant and lactating farm animals should be “DON-free”. These guidelines place significant pressures on livestock producers during periods of heavy *fusarium* pressure on feed grains. Effective solutions are required in order to enhance the utilization of *fusarium*-contaminated grains by livestock. Current options include: 1) the dilution of contaminated-grains with clean grains to achieve permissible inclusion levels; 2) the removal of the outer, more heavily contaminated portion of the grain kernel through abrasion; 3) chemical detoxification strategies, including alkaline treatment; and 4) the use of dietary additives with purported mycotoxin adsorbent properties or biological detoxification activities. Each option will have its own advantages and limitation and, beyond the use of dilution approaches, the availability of evidence-based, effective solutions for mycotoxin mitigation is currently limited. Concurrent strategies are likely required to effectively enhance the utilization of *fusarium*-contaminated grains for the livestock industry.


*Fusarium* head blight costs Canadian wheat producers $80 million annually, largely due to yield and grade loss. The presence of a few percent *Fusarium* Damaged Kernels (FDK) and the associated vomitoxin deoxynivalenol (DON) results in a significant reduction in the grade. A means to eliminate FDK would therefore have significant economic value for Canadian wheat producers. An optical method to distinguish *Fusarium* damaged kernels (FDK) from sound kernels has been developed. This method was successfully tested on 27 samples of a range of different wheat varieties grown in different locations with a classification accuracy of > 90%. A single channel classification rate of 450 kernels/second has been achieved. A pilot scale system designed to separate FDK from sound kernels at a rate of 5 tonnes per hour (50,000 kernels/second) is being constructed. The system has a singulation stage, a detection stage and a sorting stage. An average reduction in DON concentration of 86% (N=14) is observed when FDK is removed. This method has the potential to significantly reduce economic losses caused by *Fusarium*.
Effects of electron beam irradiation on deoxynivalenol in distillers dried grain and solubles and in production intermediates. T. Stepanik, D. Kost, T. Nowicki, and D. Gaba. Acsion Industries Inc., Whiteshell Laboratories, Pinawa, MB Canada, R0E 1L0, and (T.N. and D.G.) Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, MB Canada R3C 3G8.

The marketability of distillers dried grains and solubles (DDGS) is critical to the economic viability of the ethanol industry. Infection of wheat and corn feedstock with Fusarium head blight is a concern as any mycotoxins present are retained in the DDGS. To determine the effect of electron beam treatment on mycotoxin content, wheat contaminated with deoxynivalenol (DON), DDGS produced from that wheat, and three DDGS production intermediates were irradiated. The three production intermediates showed dose-dependent reductions in DON from 47.5 - 75.5 % at the highest doses used. Electron beam treatment reduced the DON level in wheat by 17.6 % at the highest dose used, but had no effect on DON in DDGS. Equipment that can treat the quantities of DDGS intermediates generated by plants producing 25-30 million gallons of ethanol/annum is commercially available. These results indicate this treatment may provide a method for reducing DON levels in DDGS on an industrial scale.
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#3. Trichothecene-chemotypes as a virulence factor in fusarium head blight of wheat. N.A. Foroud, B.E. Ellis and F. Eudes.

#4. Genetic reasons for potential chemotype shifting of *Fusarium graminearum* in Manitoba. X.W. Guo, W.G.D. Fernando, and M. Soew

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#8. Phenotypic and marker-assisted evaluation of germplasm - a starting point for wheat breeders to combat Fusarium head blight. A. Badea, F. Eudes, R. Graf, A. Laroche, and A. Gaudet


#15. Reaction of wheat genotypes from different sources to Fusarium head blight (FHB) in Manitoba, Canada. A. Malihipour, J. Gilbert, A. Brûlé-Babel, and G. Fedak Page 119


#17. Fusarium head blight of oat: Current activities of the oat breeding program at AAFC-CRC. J. W. Mitchell Fetch, A. Tekauz, B.G. Rossnagel, and M. E. Savard. Page 120


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#32. The proteomic profile of Fusarium graminearum mycelia during mycotoxin synthesis. Linda J. Harris, Audrey Saparno, Barbara Blackwell, Valar Anoop, Steve Gleddie, Danielle Schneiderman, Nicholas A. Tinker, Rebecca D. Taylor.

#33. Haplotyping of genomic regions associated with resistance to Fusarium head blight in European winter wheat. O.Kalb, G. Wenzel, M. Schmolke

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#42. Black barley as a means of minimizing the level of Fusarium head blight and deoxynivalenol contamination. T.M. Choo, B.J. Vigier, M. Savard, J.M. Yang, and J.M. Wang  

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Mycotoxins/ Mycotoxines

Update on near infrared spectroscopy as a screening tool for deoxynivalenol (DON) on whole grain and ground samples of barley. G.C. Arganosa, B.G. Rossnagel, W.G. Legge, J. Tucker, M.E. Savard, T. Zatorski, D. Voth and T. Grewal. Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada; (W.G.L. and J.T.) Agriculture and Agri-Food Canada, 18th and Grand Valley Road, Rural Route #3, Box 1000A, Brandon, MB R7A 5Y3, Canada; (M.E.S.) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada.

Deoxynivalenol (DON) reduces the quality of feed and malting barley, making it unfit for consumption. The most commonly used method to quantify DON is ELISA, which is both labour and cost-intensive. DON estimation by near-infrared (NIR) spectrophotometry offers increased screening efficiency for programs breeding for lower FHB and DON. The spectra from whole grain samples from > 5000 barley lines and ground samples from some 7000 lines grown in Fusarium Head Blight (FHB) screening nurseries at Brandon, Manitoba and Ottawa, Ontario from 2001 to 2006 have been collected by a FOSS NIR Systems 6500 near infrared spectrophotometer and merged into a product library file. Twenty percent of the spectra were chosen and placed in validation sets for each of the whole grain and ground sample sets. Remaining spectra in the product library file were used to predict the DON values of the validation sets using local equations (WinISI, Foss North America, Eden Prairie, MN). Good correlations between actual and predicted DON values for whole grain (r=0.894) and ground samples (r=0.912) were obtained, indicating that NIR spectrophotometry could be an effective screening tool, especially when the primary goal is to eliminate selections with the highest DON concentration.

Fusarium toxin (Type A and B trichothecenes and zearalenone) detection in cereals by HPLC-MS/MS. F. Berthiller1, R. Schuhmacher1, G. Buttinger1, R. Kraska1, E. Pichler2. 1Department for Agrobiotechnology (IFA-Tulln), Center for Analytical Chemistry, Christian Doppler Laboratory for Mycotoxin Research, University of Natural Resources and Applied Life Sciences, Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria; 2ROMER Labs Diagnostic GmbH, Technopark 1, A-3430 Tulln, Austria.

Contamination of food and feed with mycotoxins is a major problem worldwide. Thus, a method for the simultaneous determination of the Fusarium mycotoxins nivalenol, deoxynivalenol, fusarenon-X, 3-acetyl-deoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin and zearalenone in cereals has been developed using gradient RP-LC with atmospheric pressure chemical ionization triple quadrupole mass spectrometry (LC-APCI-MS/MS). Swift clean-up of cereal samples (maize and wheat) was performed with MycoSep® 226 columns. Quantification of deoxynivalenol, T-2 toxin and zearalenone
was performed with internal standards ($^{13}$C$_{15}$-deoxynivalenol, $^{13}$C$_{24}$-T-2 toxin and zearalanone), whereas external calibration was used for all other toxins. Detection of the mycotoxins was carried out in the multiple reaction monitoring (MRM) mode. Method performance characteristics were estimated after analysis of spiked blank maize and wheat samples. Calibration curves were linear between 10 and 1000 µg/kg and the limits of detection ranged from 0.3 to 3.8 µg/kg depending on the mycotoxin. The comparability of the method was also shown, for example LC-MS/MS and GC-ECD results for deoxynivalenol were in excellent concordance (approximately 98%). In summary, the increased analytical throughput, the use of internal standards to compensate for matrix effects to ensure more accurate and precise results and the simultaneous determination of different mycotoxins hold great promises for this and other HPLC-MS/MS methods in the near future.

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**Trichothecene-chemotypes as a virulence factor in fusarium head blight of wheat.**


In North America, three trichothecene chemotypes have been identified in *Fusarium graminearum*: 3-acetyldeoxynivalenol (3ADON), 15ADON and nivalenol (NIV). Historically, 15ADON has been the most prevalent chemotype; however, in recent years, 3ADON chemotypes have also been identified in infected crops. In addition, new strains have evolved with increased levels of 15ADON production. In order to assess the impact of chemotype change in *F. graminearum* virulence, 13 wheat genotypes were challenged with strains of these three chemotypes. Two FHB-susceptible cultivars, three resistance-expressing genotypes and eight double haploid lines generated from F1-hybrids of susceptible and resistant genotypes, were point inoculated with one of four inocula. Each inoculum consisted of a composite of strains with either low-producing 15ADON (low-15ADON), high-producing 15ADON (high-15ADON), 3ADON or NIV chemotypes. No differences were observed between treatments in susceptible or resistant genotypes, but an interaction between chemotype and disease was detected in the intermediately FHB-resistant/susceptible genotypes. A general trend of high resistance to the NIV chemotype, lower resistance to the low-15ADON chemotype, and poor resistance to both 3ADON and high-15ADON chemotypes was observed. These results suggest that chemotype may be an important factor in trichothecene virulence, and the emergence of 3ADON and high-15ADON chemotypes may represent a greater disease threat in North America.
Genetic reasons for potential chemotype shifting of *Fusarium graminearum* in Manitoba. X.W. Guo, W.G.D. Fernando, and M. Soew. *Department of Plant Science; University of Manitoba; R3T 2N2, Winnipeg, Manitoba. Canada*

The study was conducted in 15 locations, and consisted of 39 farmers’ fields sown to wheat cultivars Superb and AC Barrie, in Manitoba from 2004 to 2005. The 15 locations were further grouped into 7 regions. Percentages of 3ADON and 15ADON chemotypes of *Fusarium graminearum* (*Gibberella zeae*) ranged from 0 % to 95.7 %, and 4.3 % to 100 %, respectively. 3ADON chemotype was distributed in the south part of Manitoba. 3ADON chemotype was predominant in Sanford, Morris and Horndean; two chemotypes almost shared the same percentage in Cartier and Portage la Prairie; and 15ADON was predominant in Killarney, Souris, McAuley and Virden. The 3ADON chemotype was not found in Kenville and Dauphin. Significant gene flow of *F. graminearum* was found in southern Manitoba. There was a great variation of percentage of 3ADON chemotype within the sub grouped population from different locations and regions, which could result from a high level of genetic diversity of *F. graminearum* populations. It is suggested that sexual recombination, population age and cropping system could be associated with genetic and chemotypic diversities of *F. graminearum* populations. Wheat seed shipment and long-distance transportation of spores of *F. graminearum/G. zeae* likely contributed to the genetic migration between various places; potentially causing chemotype shifting in Manitoba.

*Biotransformation of Deoxynivalenol by Soil Microorganisms.* Jianwei He, Ting Zhou and Greg J. Boland

POSTER WITHDRAWN
**Rapid Strip Test for the Detection of Deoxynivalenol in Wheat.** Alexandra Molinelli¹, Michael Z. Zheng², Johann Binder³, and Eva Maria Binder⁴. ¹Romer Labs Diagnostic GmbH, IFA-Tulln, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria; ²Romer Labs Singapore Pte Ltd, 3791 Jalan Bukit Merah #08-08 E-Centre@redhill, SINGAPORE; ³Romer Labs Group, Technopark 1, 3430 Tulln, AUSTRIA; ⁴Erber AG, Technopark 1, 3430 Tulln, AUSTRIA.

Deoxynivalenol (DON) is a toxic natural secondary metabolite of the fungi *Fusarium graminearum* and *F. culmorum*. DON has been found predominantly in grains such as wheat, barley, oats, rye and maize. A rapid strip test called AgraStrip® Deoxynivalenol (DON) was developed to test DON in wheat quantitatively. The test is a one-step lateral flow immunochromatographic assay based on a competitive immunoassay format. DON is extracted from ground samples with deionized/distilled water and sample extracts are mixed with antibody-particle complex in microwells. A strip of AgraStrip® DON is placed into the mixed content of each microwell. The mixed content then migrates onto a membrane of the strip, which contains a test zone and a control zone. The test zone coated with DON-protein conjugate captures free antibody-colloidal gold particle complex, allowing color particles to concentrate and form a visible line. The color intensity of the test line will be inversely proportional to the concentration of DON in the sample. One line will always be visible in the control zone regardless of the presence of DON, which indicates the validity of the test. The test results are quantitatively interpreted by an AgraStrip® XReader 200. The test has a quantitation range of 250-1260ppb. It is a rapid method with a total incubation time of 5 minutes. The in-house study shows the test works well for several DON naturally contaminated wheat samples.

**AUTHORS:**
Alexandra Molinelli, Ph.D., Project Leader, ROMER Labs Diagnostic GmbH, IFA-Tulln, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria, Tel: +43 2272 66280 414, Fax: +43 2272 66280 403, e-mail: Alexandra.molinelli@romerlabs.com

Michael Z. Zheng, Ph.D., Director, Research and Development, Romer Labs Singapore Pte. Ltd, 3791 Jalan Bukit Merah #08-08, e-Centre@redhill building, Singapore 159471, Tel: +65 62755432, Fax: +65 62755584, e-mail: michael.zheng@romerlabs.com

Johann Binder, Ph.D., CEO, Romer Labs Group, Technopark 1, 3430 Tulln, Austria, Tel: +43 2272 615 33 116, Fax: +43 2272 615 33 111, e-mail: hannes.binder@romerlabs.com

Eva Maria Binder, Ph.D. Chief Scientific Officer, Erber AG, Technopark 1, 3430 Tulln, Austria, Tel: +43 2272 615 33 114, Fax: +43 2272 615 33 111, e-mail: eva.binder@erber-group.net

Deoxynivalenol (DON) is a naturally-occurring mycotoxin common in North American grains. The current Canadian guidelines for DON are under review. To support the current or revised guidelines, an analytical method validated by a multiple-laboratory collaborative study was required.

Soft wheat samples, naturally contaminated with DON at five levels, were analysed as blind duplicates by twelve laboratories in eight different countries using the subject method. Blank wheat materials were spiked in duplicate with DON for recovery assessment. The analytical portion of the sample was extracted with water. The sample extract was centrifuged, filtered, passed through an immunoaffinity column for clean-up and evaporated. The residue was dissolved in mobile phase (water + methanol [90.5 + 9.5, v + v]). The separation and determination of DON was performed by reverse-phase HPLC, with detection by UV absorption at 220 nm. The method was successfully validated and determined to be fit for use as an official method.

Resistance Breeding/ Amélioration de la Résistance

Phenotypic and marker-assisted evaluation of germplasm - a starting point for wheat breeders to combat Fusarium head blight.  A. Badea, F. Eudes, R. Graf, A. Laroche, and A. Gaudet.  Lethbridge Research Center, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, AB T1J 4B1, Canada.

Fusarium head blight (FHB) turned into a serious threat to wheat crop in Canada. Breeding resistance cultivars is an effective measure of disease control. Evaluation of genetic relationships among accessions and allele size comparison of microsatellite markers linked to quantitative trait loci (QTL) for FHB resistance allow wheat breeders to select resistance genes from different sources and to assemble them in one cultivar to increase the level of resistance. A wheat germplasm collection was evaluated by phenotypic and molecular markers analysis. Point inoculation and ELISA methods were used to assess the level of resistance and the DON concentration of germplasm collection. The genetic diversity was evaluated on the basis of Diversity Arrays Technology (DArT) markers and microsatellite markers; and allele size of the microsatellite markers linked to FHB resistance QTLs were compared between the analysed accessions and known resistance sources. The European germplasm was not closely related to other resistance sources and might be useful for pyramiding with Asian and Brazilian wheat-derived FHB resistance. The wheat cultivars Bussard and Toras were found to have a good level of resistance, a relatively low DON concentration and do not share any of the tested QTLs. These cultivars may carry novel FHB resistance genes that make them suitable candidates for further genetic studies.
MAS vs Conventional Selection for Resistance to FHB in a Wheat Backcross Breeding Program. W. Cao, G. Fedak, D. Somers, C. McCartney, A. Xue, M. Savard and H. Voldeng. *Eastern Cereal and Oilseed Research Center, Agriculture and Agri-Food Canada, Ottawa K1A 0C6; Cereal Research Center, Agriculture and Agri-food Canada, Winnipeg R3T 2M9*

A susceptible line BW 301 was crossed with a resistant line HC 374 and the F₁ backcrossed to BW 301. In the BC₁F₁, molecular markers for three genes for resistance to FHB were used to screen for resistance. Only plants with all three markers were selected. At the same time, other BC₁F₁ plants were selected (CVS), based on FHB symptoms following point inoculation in the greenhouse. In both instances, the selected plants were again backcrossed with BW 301 to produce two different BC₂F₁ populations. These two populations were evaluated for reaction to FHB reaction in the field in 2004 and 2005. The nursery was inoculated with corn and barley kernels infected with three isolates of *F. graminearum*. The means for FHB incidence and FHB severity for the MAS population were 51.9% and 32.5% in 2004, and 24.7% and 21.4% in 2005. The means for FHB incidence and FHB severity for the CVS population were 59.7% and 41.8% in 2004, and 30.4% and 34.4% in 2005. In 2006, 7 lines selected from MAS population and 20 lines (BC₁ and BC₂) from CVS population, based on performance of resistance, were used to conduct a four replication field experiment. The means for FHB incidence, FHB severity, index and DON content for the MAS lines were 32.5%, 27.1%, 9.2% and 6.7 ppm, while the means for FHB incidence, FHB severity, index and DON content for the BC₁ CVS lines were 35.4%, 30.6%, 11.6% and 9.0 ppm; for BC₂ CVS lines, 41.14%, 37.4%, 15.7% and 13.1 ppm. The best lines 2-2991 and 2-2934 with DON content of 5 ppm were selected from MAS. These results support the expectation that MAS is more efficient and effective than CVS for improvement of resistance to FHB in this wheat backcross breeding program.

Enhanced Fusarium Head Blight Resistance in Bread Wheat and Durum by Alien Introgressions. G. Fedak¹, W. Cao¹, A. Xue¹, M. Savard¹, J. Clark², D.J. Somers³, H.D. Voldeng¹, and J. Gilbert³. *Eastern Cereals and Oilseeds Research Center, Building 50, AAFC, 960 Carling Avenue, Ottawa, Ontario K1A 0C6 Canada; Semiarid Prairie Agricultural Research Center, AAFC, P.O. Box 1030, Swift Current, Saskatchewan, S9H 3X2 Canada; Cereal Research Centre, AAFC, 195 Dafoe Road, Winnipeg, Manitoba, R3T 2M9 Canada*

Fusarium head blight has become a devastating disease of cereals in temperate climate regions of the world. There appears to be sufficient inoculum built up so that the occurrence of rainfall during flowering of the crop is certain to cause extensive infection. The single best source of resistance under our conditions has been the Chinese variety Sumai3. However, under intensive infection pressure in our epiphytotic nursery, Sumai3 will suffer up to 20% floret infection and vomitoxin =deoxynivalenol (DON) content as high as 5.0ppm. Therefore, we began a research for additional sources of resistance. Lines with resistance obtained from *T. monococcum* (AA) and *Ae. speltoides* (BB) are at the most advanced stage of development. Homozygous lines were grown in replicated yield trials in 2006 and 2007. The protein contents of the two lines were 14.9% and 16.8%
respectively. Their FHB index was equal to the best lines of breeding material in the test. For other agronomic traits such as test weight, TKW, height and rust resistance, the lines were equal to the checks, i.e. there is virtually no linkage drag from the donor parents. Homozygous lines have now been produced with resistance derived from *T. timopheevi* (AG), *Ae. cylindrica* (CD) and *T. miguschovae* (AGD) and will be evaluated in replicated yield trials in the next growing season. Additional resistance has now been found in *Hordeum californicum* and its transfer into bread wheat has been initiated. In the meantime, molecular markers are being developed to be used to pyramid the various sources of resistance and incorporate them into contemporary cultivars. In the case of durum wheat, high levels of resistance have not been found in the species itself. Alien sources of resistance for durum FHB improvement have been found in *T. carthlicum* (AB), *T. miguschovae* (AGD), *Tritordeum* (ABH) and *Th. elongatum* amphiploids (ABE). The *T. carthlicum* resistance has recently been mapped to chromosome 2B. The range of DON levels in *Tritordeum* derivatives was 0.6-11.3 ppm, where the level in cultivar Strongfield was 14.1 ppm, in field plots in 2006. Attempts are underway to incorporate the resistance from the E genome into durum wheat. Disomic addition lines (2n=30) with resistance have now been isolated.


Fusarium head blight (FHB) is one of the most serious diseases challenging the wheat industry in Canada. Since the 1980 epidemic in eastern Canada, and that of 1993 in Manitoba, efforts to breed for FHB resistance have been intense. The disease is strongly influenced by the environment, thus the use of multiple testing sites across Canada to examine new germplasm or wheat lines for reaction to FHB can facilitate the identification of FHB-resistant lines that are adapted to one or more regions of the country. An East-West nursery was established for this purpose. The check lines comprise wheat cultivars with early/late maturities, resistant/susceptible FHB reactions, and adaptation to either eastern or western Canada. The checks (~10) have been constant over the past 8 years. The reliability of the reactions of new entries can be judged by the consistency of the checks’ reactions. Using biplots to examine genotype X environment interactions we have learnt that some environments (year, location) differentiate between entries better than others, but all locations have differentiated better in one or more years than others, indicating both the advantages of and necessity for multi-site testing. The FHB reactions of the most resistant and most susceptible checks have been consistent across all environments.
Field Evaluation of Fusarium Head Blight Resistance in German and Canadian Spring Wheat Germplasm. G. Humphreys, D. Simard, A. Brûlé-Babel, H. Voldeng, L. Tamburic-Ilinic, L. Hartl, J. Haebeler, E. Ebmeyer, D. Brown and D. Somers. Cereal Research Centre, AAFC, 195 Dafoe Road, Winnipeg, MB R3T 2M9; (A. B-B.) Dept. of Plant Science, 66 Dafoe Road, University of Manitoba, Winnipeg, Manitoba, R3T 2N2; (H.V.) Eastern Corn and Oilseeds Research Centre, AAFC, Central Experimental Farm, Ottawa, Ontario K1A 0C6; (L. T-I.) Dept. of Plant Agriculture, University of Guelph, Ridgetown Campus, ON; (L.H. and J.H.) Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Vöttingerstrasse 38, D-85354 Freising, Germany; (E.B.) Lochow-Petkus GmbH, Bollersener Weg 5, D-29303 Bergen, Germany.

Fusarium head blight (FHB) is a fungal disease of wheat with significant economic impact for the grain industry. Control of the disease includes foliar applied fungicides and genetic resistance; however, control of FHB with fungicides has been unreliable. The objective of this study was to evaluate the reaction of Canadian and German wheat germplasm to FHB. Through the evaluation of germplasm developed using different breeding strategies in alternative environments, new sources of FHB resistance may be identified. Twenty-six breeding lines from Canada and Germany, 3 resistant and 2 susceptible checks were screened for FHB resistance at 3 sites in Canada and 2 sites in Germany in 2007. In Canada, lines were scored using a visual rating index (VRI), while in Germany, FHB was evaluated as mean incidence scored multiple times (4-5) during the season. VRI was significantly correlated with the German incidence rating. HY687 (Canadian) and LP819.4.04 (German) had the lowest FHB ratings in both Canada and Germany. In general, the lines evaluated behaved similarly, regardless of continent. However, W984-8767 (Canadian) had a FHB rating 2.6 times higher in Germany than in Canada, and Kadrilij (German) had a FHB rating 2.4 times higher in Canada than in Germany. Thus, for some lines genotype x environment effects appear to be important.

First results of screening of Triticum monococcum for resistance to Fusarium culmorum. D. Kopahnke¹, T. Miedaner², V. Lind¹ and E. Ordon¹. ¹Federal Centre for Breeding Research on Cultivated Plants, Institute of Epidemiology and Resistance Resources, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany, ²State Breeding Institute, University of Hohenheim. Fruwirthstr. 21, 70593 Stuttgart, Germany

In order to broaden the genetic base of resistance of Triticum aestivum to Fusarium head blight, 84 accessions of Triticum monococcum were analysed for resistance to Fusarium culmorum in field trials at Quedlinburg (Saxony-Anhalt, Germany) and at Hohenheim (Baden-Württemberg, Germany) in the growing period 2006/2007. Triticum monococcum was chosen as a possible donor of resistance due to the fact that it proved in prior investigations to be a useful source for resistance to Puccinia triticina, Pyrenophora tritici-repentis and Blumeria graminis. The genotypes sown in double rows were arranged in a block design with two replications. At full flowering, each accession was spray inoculated with the aggressive Fusarium culmorum isolate FC46 (1x10⁶ Conidia/ml) and AUDPC was calculated based on four observations. The correlation between the results obtained at Hohenheim and Quedlinburg was 0.796 (P = 0.01). The
analysis of variance revealed significant genotype and location effects as well as a significant genotype x location interaction. Based on these results, 28 T. monoccocum accessions were identified significantly better performing than the resistant T. aestivum check cultivar 'Toras'. Respective T. monoccocum accessions will now be analysed in detail in growth chamber experiments.

Two-row malting barley breeding lines with improved resistance to fusarium head blight. W. G. Legge, J. R. Tucker, B. Bizimungu, A. Tekauz, M. E. Savard, B. Vigier and R. A. Martin. Brandon Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1000A, R.R. 3, Brandon, MB R7A 5Y3, Canada; (B.B.) Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, AB T1J 4B1, Canada; (A.T.) Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada; (M.E.S., B.V.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada; (R.A.M.) Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, PE, C1A 4N6, Canada.

Fusarium head blight (FHB) incited by Fusarium graminearum Schwabe is presently the most significant disease of barley in Canada, primarily due to mycotoxin contamination of grain and quality degradation. Development of FHB resistant cultivars with low deoxynivalenol (DON) accumulation is an important objective of the two-row malting barley breeding program at Agriculture and Agri-Food Canada, Brandon, MB. Breeding strategies include doubled haploid production, in vitro selection, use of exotic FHB resistance sources and screening for transgressive segregants from standard breeding crosses. Breeding lines are evaluated over several years in an irrigated FHB nursery at Brandon, and in subsidiary FHB nurseries at Charlottetown, PE, Ottawa, ON, Glenlea, MB and Portage la Prairie, MB. TR05915, developed by subjecting plants of barley cultivar CDC Kendall to in vitro selection, has 25% lower DON content than CDC Kendall. Utilization of exotic sources has been problematic due to negative effects on malting quality. However, several FHB resistant lines from cross BM9856D involving the two-row Chinese line Harbin will serve as excellent parents. Three two-row malting lines (TR07298, TR07299 and TR07201) with low DON accumulation from standard breeding crosses were advanced to the 2007 Western Cooperative Two-row Barley Registration Test. A new two-row malting barley cultivar with improved FHB resistance may be registered within 3 years.
Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most important diseases of wheat. Evaluation of reaction of wheat to disease is essential for host-pathogen interaction studies, identification of resistant germplasm for breeding programs, and disease management. In this study, 63 genotypes from Canada, Iran and CIMMYT (Mexico) were tested in two nurseries in Manitoba, Canada for two years with spray inoculation. FHB incidence, severity, and index were assessed 21 days after inoculation and Fusarium-damaged kernels (FDK) were determined after harvest. Results showed that the genotypes differed significantly for disease incidence, severity, index, and FDK at $\rho \leq 0.001$. The effect of environment on disease incidence and FDK was significant. All traits were significantly affected by the year. Genotype x environment, genotype x year, and genotype x environment x year interactions had significant effects on all traits, but the effect of environment x year was not significant on disease incidence and index. There was a high positive correlation among disease incidence, severity, and index, but these had a relatively lower correlation with FDK. These results will be used to choose a group of genotypes with a range of reactions to the disease for a host-pathogen interaction study being conducted with *F. graminearum* isolates from the aforementioned countries.


The wheat leaf rust resistance locus *Lr34* is known to impart resistance to a wide range of diseases including stripe rust, barley yellow dwarf virus, and stem rust. Populations were created to study the effect of *Lr34* on FHB resistance in wheat. The FHB resistant cultivar ‘Sumai 3’, which has *Lr34*, was crossed to the leaf rust susceptible cultivar ‘Thatcher’ and an F2 plant that lacked *Lr34* was backcrossed to ‘Sumai 3’. This process was done repeatedly to create a BC2 F3 population that segregated for *Lr34*. A pair of sister lines, one homozygous for *Lr34*, the other homozygous for the susceptible allele were selected from each of 38 families. These paired sister lines were grown in *Fusarium graminearum*-inoculated field nurseries in 2006 and 2007 and a growth cabinet evaluation. *Lr34* had no significant effect on FHB as all lines were highly resistant, similar to ‘Sumai 3’. For leaf rust, the lines with *Lr34* ranged from 0-10% flag leaf coverage by pustules while those without *Lr34* ranged from 50-80%.
Fusarium head blight of oat: Current activities of the oat breeding program at AAFC-CRC. J. W. Mitchell Fetch, A. Tekauz, B.G. Rossnagel, and M. E. Savard. (J.M.F., A.T.) Cereal Research Centre(CRC), Agriculture and Agri-Food Canada, Winnipeg MB, R3T 2M9; (B.G.R.) Crop Development Centre, University of Saskatchewan, Saskatoon SK, S7N 5A8; (M.E.S.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada

Fusarium head blight (FHB), incited by Fusarium graminearum Schwabe and other Fusarium spp., was recognized as a prevalent disease of oat in Manitoba in 2002 when an initial field survey was conducted. Subsequent surveys and the isolation of FHB-causing species from the sampled oat grain indicated that FHB has become an important disease of oat, especially in the eastern prairies. Therefore, suitable sources of genetic resistance need to be identified for use in oat breeding programs. This is being accomplished through the evaluation of advanced breeding lines and exotic oat accessions in a mist-irrigated artificially-inoculated FHB Nursery at Portage la Prairie, Manitoba, first established in 2003. Several lines with lower levels of Fusarium on the seed as evaluated through fungal growth on potato dextrose agar (PDA) plates and/or lower levels of accumulated deoxynivalenol (DON) have been identified, which are being currently utilized in the crossing program at CRC. These crosses could potentially result in an FHB resistant cultivar within 8-10 years.


Fusarium head blight (FHB), caused by Fusarium graminearum Schwabe, is a significant barley disease in Canada. The Crop Development Centre (CDC) barley improvement program at the University of Saskatchewan participates in the collaborative FHB screening nursery project initiated in 2000 at AAFC, Brandon, MB. Screening several thousand CDC lines from 2001 to 2007 has identified five hulled selections consistently demonstrating lower DON accumulation comparable or better than the resistant check CI4196. An agronomically promising hulled line, SB00106 (TR04378), showed low DON levels over all years and test sites from 2001 through 2005. This line was released as a new cultivar named CDC Mindon in 2006 for cultivation in western Canada and replaced CI4196 as the resistant check in the Brandon FHB nursery in 2005. Hulless lines, in general, show lower DON accumulation and 17 CDC selections with consistently lower FHB and DON accumulation over years and locations have been identified. A high portion of waxy starch hulless lines show resistance and lower DON accumulation in the greenhouse and field. In addition to strict field screening at Brandon, field and greenhouse screening of two barley populations (CDC Helgason/CI4196 and TR360/2ND16092) identified lines showing resistance to FHB and DON accumulation indicating that FHB resistant lines with acceptable DON accumulation could be selected.
In conclusion, notable progress has been made in identification of barley lines with reduced DON accumulation and resistance is being transferred to elite barley lines.

CDC Mindon 2 Row Barley with low DON accumulation  B.G. Rossnagel, T. Zatorski, W.D. Voth, G.J. Scoles, W.G. Legge, J.R. Tucker, A. Tekauz, and M. Savard. Crop Development Centre, Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada, S7N 5A8; (W.G. L., J.R.T.), Agriculture and Agri-Food Canada, Brandon Research Centre, P.O. Box 1000A R.R.#3 Brandon, Manitoba, Canada, R7A 5Y3; (A.T.) Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Rd. Winnipeg, Manitoba, Canada, R3T 2M9; ( M.S.) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Ave., Ottawa, Ontario, Canada, K1A 0C6.

CDC Mindon (CN107350; CFIA (Canadian) Reg. No. 6224, Canadian PBR Appl. No. 07-5903) is a two-rowed spring feed barley (Hordeum vulgare L.) developed at the Crop Development Centre (CDC), University of Saskatchewan, Saskatoon, Saskatchewan, with extensive collaboration at the Fusarium Head Blight (FHB) (Fusarium graminearum) /deoxynivalenol (DON) screening stage by Agriculture and Agri-Food Canada (AAFC) at the Brandon Research Centre, Brandon, Manitoba, and the Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Ontario. CDC Mindon was tested in CDC yield trials as SB00106 in 2000 – 2003 and in the Western Canadian Cooperative Two-Row Barley Registration trial as TR04378 during 2004 and 2005. CDC Mindon was registered for production in western Canada as it has demonstrated good agronomic performance combined with good kernel quality and, of greater significance, enhanced resistance to FHB and lower DON accumulation in combination with tolerance to spot blotch (Cochliobolus sativus). CDC Mindon is also resistant to the barley smuts. It was registered on February 23, 2007 by the Variety Registration Office of the Canadian Food Inspection Agency, Ottawa, Ontario, Canada.

CDC Mindon originates from the cross TR339/TR251 made at the CDC, University of Saskatchewan, Saskatoon, Saskatchewan, in 1996 and was developed using single seed descent. TR339 is a two-rowed breeding line developed at the CDC from the cross CDC Dolly (Rossnagel and Harvey, 1994) by WM873-27, a breeding line jointly developed by the AAFC Cereal Research Centre (CRC), Winnipeg, Manitoba, and the Brandon Research Centre. TR251 is a two-rowed breeding line developed at the Brandon Research Centre from the three way cross of TR229, a two-rowed breeding line from the Brandon Research Centre, AC Oxbow (Government of Canada, 2007) and ND7556, a two-rowed breeding line from North Dakota State University, Fargo, North Dakota, USA.
Fusarium head blight resistance assessment of spring wheat in Hokkaido, Japan.
N. Sato, K. Nakamichi, Y. Yoshimura. Kitami Agricultural Experiment Station, 52 Yayoi, Kunneppu-choy, Tokoro-gun, Hokkaido, 099-1496, Japan.

Fusarium head blight (FHB) caused by Fusarium graminearum (Schwabe) is one of the most serious diseases of spring wheat in Hokkaido. It is important to improve the resistance to FHB. We evaluated the FHB resistance of wheat breeding lines and cultivars in irrigated, inoculated nurseries over two years (2006-2007). Results suggested that the FHB symptoms of wheat lines were dependent on flowering dates and daily weather conditions. Therefore, check varieties within each flowering period should be used during FHB resistance assessment. In order to develop resistant lines, Sumai3 and its progeny were crossed to adapted Japanese lines. We evaluated FHB severity in progeny derived from these crosses and some highly resistant lines were selected. Lines derived from the cross Sumai3 x OS68-2 (high resistance to pre-harvest sprouting) showed high levels of resistance to both FHB and pre-harvest sprouting.

Development of winter durum wheat cultivars for Ontario and estimate of the deoxynivalenol (DON) level in potential candidate cultivars. L Tamburic-Ilinic and C. A. Griffey. Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, ON; (C. A.G) Virginia Tech University, 334 Smyth Hall, Blacksburg, VA.

Fusarium head blight (FHB) is an important disease of common and durum wheat. Durum wheat is used for pasta production and has the potential to be established as a new class of wheat in Ontario. However, at present, there is no winter durum registered in Ontario. In general, in most evaluations, durum wheats have shown lower levels of FHB resistance than bread wheats. The objective of this study was to estimate the level of DON accumulation and agronomic performance of winter durum lines from the Virginia Tech University breeding program. Eleven winter durum lines were tested for DON accumulation after spray-inoculation with F. graminearum in Ridgetown, ON and Blacksburg, VA. In addition, the lines were tested for agronomic performance in two locations in Ontario (Ridgetown and Centralia). Differences in DON level amongst the cultivars were identified in both FHB nurseries after inoculation. In 2007, DON levels ranged from 0.5 ppm to 7.0 ppm and from 0.6 ppm to 5.3 ppm in harvested grain from Ridgetown and Blacksburg, respectively. The highest yielding cultivar in Ridgetown was VA05WD-31 and in Centralia VA05WD-39. Additional winter durum lines will be tested in Ontario in 2007-2008 with the goal of registering the best performing ones for commercial production in Ontario.
An update on the development of Fusarium Head Blight (FHB) resistant wheat germplasm and cultivars with lower deoxynivalenol (DON) accumulation at the University of Guelph. L Tamburic-Ilincic, D. E. Falk, and A.W. Schaafsma Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, Ontario, N0P 2C0; (D.E.F.)Department of Plant Agriculture, University of Guelph, 50 Stone Road. E., Guelph, Ontario, N1G 2W1

Fusarium head blight (FHB), caused by Fusarium graminearum (Schwabe), is an important wheat disease. The wheat breeding program at the Ridgetown Campus, University of Guelph has participated in the collaborative Northern Uniform Winter Wheat Scab Nursery (NUWWSN) with other breeders from USA for the past five years. The other partners include University of Arkansas (AF), Cornell University, University of Illinois, Virginia Tech University, The Ohio State University (OH), University of Kentucky, University of Missouri, University of Nebraska, Michigan State University (MI), Purdue University, and the University of Maryland. Each partner has been contributing six wheat lines which are tested across all locations every year. Ridgetown location usually belongs to the same mega environment (produces similar genotype rankings and results) as OH, MI and AF. In 2002-2003, our line named RCATL33 (FHB resistance derived from both Sumai 3 and Frontana) was rated amongst some of the most resistant entries in the test. In the 2006 NUWWSN Test, RCAT TF203/2 was among the best entries for FHB and DON, in addition to excellent soft wheat quality traits (http://www.scabusa.org -NUWWSN Reports). Our goal is to release winter wheat germplasm and cultivars, adapted to Ontario, with improved FHB resistance, quality, and yield. Our current results and strategies to achieve this goal will be discussed.

Fusarium head blight resistance and deoxynivalenol accumulation in wheat developed by the anther culture method with trichothecenes added in the induction medium. L. Tamburic-Ilincic and F. Eudes Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, Ontario, N0P 2C0; (F. E.) Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, Alberta, T1J 4B1

Fusarium graminearum (Schwabe) causes Fusarium head blight (FHB), an important wheat disease, and produces the mycotoxins in grain. Deoxynivalenol (DON) is the most frequent mycotoxin in wheat grain in Canada. Different types of FHB resistance have been reported in wheat; one type of resistance alone is not sufficient to prevent severe FHB epidemics. Selection for FHB resistance and lower DON content is an important goal of cereal breeding programs. We produced double haploid (DH) lines by anther culture method, and simultaneously screened them for tolerance to mycotoxins by adding a mixture of trichothecenes (DON, 15-o-ADON, Nivalenol and T-2 toxin) to the induction medium. The plants that survived the trichothecene mixture were inoculated with F. graminearum at anthesis, and were rated later for visual symptoms of FHB infection. In mature grain, the DON content was determined for each line using a fluorometric test-FluoroQuan (Romer Labs Inc, Union, MO). The level of DON among the lines ranged from 0.2 to 3.7 ppm in 2006, and from 1.1 to 5.1 ppm in 2007. Our results indicate that depending on parents used in crosses, adding trichothecenes in vitro
was useful for selecting lines with lower potentials of DON concentration. The advantages of using early screening for mycotoxins tolerance and DH techniques will be discussed.

**Type 1 and Type 2 Resistance to Fusarium Head Blight in Wheat – A True or False Distinction?**  J. Thomas, D. Somers and P. Cuthbert. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB R3T 2M9. PC: Box 667, 65-3rd Street NE, Carman, MB R0G 0J0.

A strong and persistent correlation between disease incidence and disease severity was detected in wheat (*Triticum aestivum* L. and *Triticum turgidum* L.) for Fusarium Head Blight (FHB) across a range of genotypes differing widely in the level of their resistance. Modelling of FHB resistance (incidence and severity) showed that independent effects for resistance to infection (Type 1) and resistance to disease spread (Type 2) cannot explain this pattern. Although the model showed that incidence and severity were necessarily weakly correlated, the observed correlation was too strong for the assumption of independence between resistance to infection and resistance to spread to be plausible. Instead the close relationship between incidence and severity requires that, across a wide range of genotypes, increasing resistance is accompanied by correlated and proportionate increments in both types of resistance. Joint effects for resistance to infection (Type 1) and resistance to spread (Type 2) were also observed in the expression of the resistance gene *Fhb2* based on the evidence of random inbred lines segregating for this gene. It was suggested that combined Type 1 and Type 2 effects are a common feature in the expression of genes affecting FHB resistance in wheat.

**Assessment of Fusarium Head Blight Resistance and Deoxynivalenol Content in Winter and Spring Wheat.** Y. Yoshimura, N. Sato, S. Kobayashi, T. Yamana. Kitami Agricultural Experiment Station, 52 Yayoi, Kunneppu-ryo, Tokoro-gun, Hokkaido, 099-1496, Japan.

Fusarium head blight (FHB) is a devastating disease in cereals, resulting in yield loss and contamination of harvested grains with mycotoxins, including deoxynivalenol (DON). Wheat breeding lines and cultivars with various levels of resistance to FHB were evaluated over three years (2004-2006) in an irrigated nursery inoculated with *F. graminearum*. In 2006, irrigation of the spring wheat nursery was not required because high humidity and temperature at flowering time resulted in high DON accumulation. FHB severity was assessed at 21 days after anthesis. DON was measured using the ELISA technique. DON content was positively correlated with Fusarium damaged kernels (FDK) in winter and spring wheat, but FHB severity was not a good predictor of DON level and FDK in harvested grain. Nevertheless, lines with low DON content can be indirectly achieved by selecting for reduced FHB severity and FDK across years or environments. FHB severity among lines compared over years (2004-2006) was highly correlated but DON content ranged from low to high. DON accumulation is highly
influenced by the environment. Further research is required into the mechanism(s) of reduced DON accumulation and methods to assess and select for low DON lines.

Physiology of Resistance/ Physiologie de la Résistance

Characterization of an alien source of resistance to FHB transferred to Chinese Spring wheat. S. S. Miller, T. Ouellet, G. Fedak, E. Watson, J. Lazebnik and J.-R. Wang. Eastern Cereal and Oilseed Research Centre, Agriculture & AgriFood Canada, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada

To characterize novel sources of resistance to *Fusarium graminearum* Schwabe we compared Chinese Spring (CS), a wheat variety susceptible to *Fusarium*, to CS-7E, a wheat line derived from CS containing the 7E chromosome from the wild grass *Thinopyrum elongatum* (Host) D. R. Dewey, and CS-7ES, also derived from CS, containing only the short arm of the 7E chromosome, both of which carry resistance to *Fusarium*. To study fungal growth, we used a strain of *F. graminearum* that was transformed to express green fluorescent protein from jellyfish. Clear differences between the CS-7E and CS-7ES lines and the parent line CS were observed. Growth and spread of the fungus occurred earlier and to a greater extent in CS than in CS-7E and CS-7ES. In CS, after infiltration of the inoculated floret, the fungus progressed both up and down inside the rachis as many as 3 nodes from the point of inoculation. In both CS-7E and CS-7ES, the fungus did not progress into the rachis beyond the node of the inoculated floret, and appeared to be contained at the point of inoculation. At the molecular level, both up- and down-regulation of genes was observed after inoculation. Examples from the shikimic acid pathway will be presented.

Genomics and Genetics/ Génomique et Génétique

A multi-stress selection approach with better biodiversity of resistance mechanisms achieves good results for the development of Fusarium resistant germplasm - the example of FL62R1 wheat. A. Comeau, F. Langevin, J. Gilbert, H. Voldeng, M. Savard, Y. Dion, S. Rioux, R.A. Martin, S. Haber, D. Somers. SCRDC, Agriculture and Agri-Food Canada, 2560 Hochelaga, Québec, QC, G1V2J3; (J.G., S.H., D.S.) CRC, Agriculture and Agri-Food Canada, 195 Dafoe Rd, Winnipeg, Man., R3T2M9; (H.V., M.S.) ECORC, Agriculture and Agri-Food Canada, Ottawa, ON, K1A0C6; (Y.D., S. R.) CEROM, 740, chemin Trudeau, Saint-Mathieu-de-Beloeil, Québec, QC, J3G 4S5; (R.A.M.) CLRC, Agriculture and Agri-Food Canada, 440 University ave, Charlottetown, PEI, C1A4N6.

The quantitative nature of FHB resistance is accepted as a fact of life. Many other plant traits are quantitative, regulated, and tend to correlate with each other. The feasibility of pyramiding FHB resistance genes with a series of other resistance and agronomic traits
was assessed. Higher biodiversity of parental germplasm was combined with a stress complex involving combined inoculation with FHB, BYDV, plus many optional stresses, (drought, waterlogging, mineral deficiencies, rusts, mildew, root rot). Stress was used on F<sub>1</sub> to F<sub>5</sub> generation plants. Approximately, 5,000 to 10,000 F<sub>1</sub> plants per year were evaluated, primarily from 4-way crosses (F<sub>1</sub>/ F<sub>1</sub>), and 95-99% of lines were discarded per generation, based on resistance responses. A few wheat lines were obtained that withstood most biotic and abiotic stresses. The F<sub>9</sub> line FL62R1 has resistance to most of the diseases present in Eastern Canada (FHB, rusts, mildew, BYDV and leaf spots) combined with good yield potential based on preliminary trials. In point inoculation trials with *Fusarium graminearum*, FL62R1 is somewhat inferior to Sumai 3; however in FHB-inoculated field trials, FL62R1 had FHB resistance similar to Sumai 3 (based on spike symptoms or FDK). With the availability of new parents like FL62R1 and using the muti-stress system, the creation of useful FHB resistant gemplasm should be easier in the future.


Microarray analysis was performed on spikelets of point inoculated wheat heads of one fusarium head blight (FHB) -susceptible cultivar (Superb) and two FHB-resistant double haploid lines (DH1 and DH2). DH1 and DH2 were generated from crossing and backcrossing resistant cultivars CIMMYT11 and CM82036, respectively, with Superb. DH1 and DH2 each share 75% genetic identity with Superb, and exhibit good resistance to initial infection (Type I, DH1) or disease spread (Type II, DH2). Five inoculums, GZ3639 (*Fusarium graminearum* strain), GZT40 (trichothecene non-producing GZ3639 mutant), GZT40 supplemented with deoxynivalenol trichothecene, deoxynivalenol, and water, were used to inoculate the central florets during anthesis. Spikelets above and below the inoculation point were collected at 3, 8 and 24 hours after inoculation (hai) for transcriptome comparison of systemic changes and constitutive differences in resistant versus susceptible genotypes. The most striking difference observed was higher transcription of a lipid transfer protein (LTP) -3 in DH1 across all treatments and harvest times. LTP-1 and thaumatin expression increased in DH2 by 8 hai, but were lower than in DH1 and Superb at 3 and 24 hai. Thionin-like expression was lower in both resistant lines, except at 8 hai where expression increased in DH1 to the same levels as Superb.
Analysis of resistance to FHB in ‘Sumai-3’ and two susceptible near isogenic lines: Morphological responses to the disease and identification of quantitative and qualitative differences at the gene expression level. S. Golkari\(^1\), J. Gilbert\(^1\), T. Ban\(^2\), S. Prashar\(^1\) and J.D. Procunier\(^1\). 1. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB R3T 2M9; 2. Division of Plant Genetic Resource Sciences, Kihara Institute for Biological Research, Yokohama City University.

Fusarium head blight, predominantly caused by *Fusarium graminearum* (Schwabe), is a destructive disease posing a serious threat to wheat production worldwide. cDNA microarrays representing wheat ESTs were used to investigate QTL-specific differential gene expression between the resistant cultivar ‘Sumai-3’ and susceptible near isogenic lines (NILs), differing in 3BS and 2AL QTL regions, following inoculation with *F. graminearum*. Data analysis revealed significant differences in the level of resistance between ‘Sumai-3’ and the susceptible NILs and in the level of disease caused by spray (SI) and point (PI) inoculation methods. The significant interaction of inoculation method x genotypes suggested that SI and PI may target different types of resistance to FHB in wheat. A total of 56 ESTs representing 25 unigenes responded significantly to *F. graminearum* infection. Genes encoding pathogenesis-related proteins such as β-1,3-glucanase, chitinase, wheatwins and thaumatin-like proteins were significantly induced, and infection triggered an overall suppression of photosynthetic activity in infected tissues. Differences in gene expression between the resistant Sumai-3 and the susceptible NILs were found to be mainly quantitative in nature. Qualitative differences were found for one gene related to the phenylpropanoid pathway and one gene of unknown function.

Predicted chromosome bin map location of wheat unigenes, differentially expressed in response to *Fusarium graminearum* infection. S. Golkari, J. Gilbert, S. Prashar and J.D. Procunier. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB R3T 2M9;

Comparative mapping based on the conserved genome organization between wheat and the model plant, rice, facilitated transferring structural genomic information between these closely related grass species. In this study, the predicted physical map location of unigenes responsive to *Fusarium graminearum* infection was determined based on the existing micro-synteny between the wheat and rice genomes using an *in silico* mapping procedure. Unigenes encoding β-1,3-glucanase which are significantly induced in response to *F. graminearum* infection were correctly positioned into deletion bins on the long arm of chromosome 3B of wheat. This finding was in agreement with the previously reported genetic and physical map positions for the β-1,3-glucanase genes. This study demonstrates the value of using micro-synteny between wheat and closely-related species in localizing unmapped wheat ESTs that are functionally associated with FHB resistance. The ESTs representing unigenes that responded significantly to *F. graminearum* infection provided a source of functional candidate genes for developing new and effective markers that are both physically and functionally associated with FHB resistance in wheat.
Mapping of QTL for Resistance against Fusarium Head Blight in the Winter Wheat Population Pelikan//Bussard/Ning8026. J. Häberle¹, J. Schondelmaier², G. Schweizer¹, G. Zimmermann¹, L. Hartl¹. ¹Bavarian State Research Center for Agriculture, Vöttinger Str. 38, 85354 Freising, Germany, ²Saaten Union Resistance Laboratory GmbH, Hovedisser Str. 92, 33818 Leopoldshöhe, Germany

Molecular markers for resistance QTL (Quantitative Trait Loci) against Fusarium head blight (FHB) enable early selection in breeding programs. Marker-assisted pre-selected populations should be enriched with resistance genes and still possess enough phenotypic variation for the selection of superior lines in further, classical breeding steps.

The aim of this study was the identification of FHB resistance QTL of the donor Ning8026 in the background of elite breeding material adapted to the European climate. The mapping population consisted of 122 recombinant inbred lines. FHB resistance was evaluated following spray inoculation with Fusarium culmorum in five environments.

Molecular mapping was conducted using microsatellites and AFLP markers. With a multiple interval mapping method two major resistance QTL were identified on chromosomes 5BL/7BS (R² = 19%) and 6BS (R² = 15%). The resistance QTL on 5BL/7BS overlapped with QTL for plant height and heading date.

In the same regions QTL were mapped derived from Sumai 3 (Yang et al., 2003; Cuthbert et al., 2007) and Wangshubai (Jia et al., 2005). Now both QTL were introgressed from the donor Ning8026 in central European breeding material and proved to be effective in field trials. Since the donors of the QTL have not been utilized in European breeding material, well-adapted lines of the mapping population are available as donors for marker-assisted breeding programs.

References:

The proteomic profile of Fusarium graminearum mycelia during mycotoxin synthesis. Linda J. Harris, Audrey Saparno, Barbara Blackwell, Valar Anoop, Steve Gleddie, Danielle Schneiderman, Nicholas A. Tinker, Rebecca D. Taylor. Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada.

We are using a proteomics approach to identify Fusarium graminearum (Gibberella zeae [Schwein.] Petch) genes, proteins, and pathways important for successful host invasion. We chose to monitor proteomic changes in F. graminearum cells grown in aseptic liquid
culture conditions conducive to trichothecene production in the absence of contaminating plant proteins. Three biological replicates of a time course study were subjected to non-gel-based quantitative iTRAQ proteomic analysis. Statistical analysis of a filtered dataset revealed 130 *F. graminearum* proteins that exhibited significant changes in expression, of which 72 (including predicted secreted proteins, homologs of other fungal virulence proteins) were up-regulated relative to their level at the initial phase of the time course. There was good agreement between up-regulated proteins identified by 2-D PAGE/tandem mass spectrometry and iTRAQ. Finally, as confirmation of the relevance of our experimental design to in vivo plant infection, 99% of the transcripts of the proteins determined to be significantly up-regulated by iTRAQ analysis were also identified as being expressed under infection conditions in planta. Numerous candidate pathogenicity proteins were identified using this technique. These will provide leads in our search for mechanisms of host invasion and novel antifungal targets. We are currently disrupting respective gene candidates in the fungus to explore function.

**Haplotyping of genomic regions associated with resistance to Fusarium head blight in European winter wheat.** O.Kalb, G. Wenzel, M. Schmolke. Technische Universitaet Muenchen, Institute of Plant Breeding, Am Hochanger 2, D-85350 Freising, Germany

Fusarium head blight (FHB) caused by *Fusarium culmorum* is a destructive disease of winter wheat in Germany. So far only a few winter wheat resistance sources have been characterized by molecular markers, like the French cultivar Renan and the Romanian genotype F201R. For Renan two major resistance quantitative trait loci (QTL) are mapped on chromosome 5A and for F201R major FHB resistance QTL are located on chromosomes 1B and 5A (Gervais et al. 2003, Shen et al. 2003). QTL mapping usually involves the use of segregating populations derived from contrasting parents. A complementary method to identify resistance QTL is association mapping which also provides useful haplotypes for marker assisted selection. In order to perform haplotype analyses and to characterize allelic variation, a set of 190 European winter wheat cultivars were genotyped with SSR markers linked to known QTL for FHB resistance and evaluated in field trials at four locations in 2007. Preliminary results based on 20 SSR markers and 60 cultivars confirm a high level of allelic variation for FHB resistance loci in winter wheat. None of the 60 cultivars showed the same haplotype compared to F201R or Renan. Further microsatellite analyses for haplotyping of additional QTL regions as well as association mapping are under way.

References:
Characterization of defensin gene PDC1 from maize (Zea mays L.) and its protein antifungal activity against Fusarium graminearum. Pragya Kant, Wen-Zhe Liu, Pat Masliamany, Jia-Zheng Yuan, Jie Liu, and K. Peter Pauls. Department of Plant Agriculture, University of Guelph, ON, Canada, N1G 2W1

Plant defensins are small antimicrobial, basic peptides that have a characteristic three-dimensional folding pattern which is stabilized by eight disulphide-linked cysteines. We have cloned and characterized a maize defensin gene (PDC1) and have shown that it has antifungal activity. A 545 bp fragment was amplified from genomic maize DNA with the primers designed from a barley defensin EST. The cloned genomic DNA fragment sequence contains a 249 bp open reading frame, a 102 bp intron, and a 194 bp untranslated region. The deduced PDC1 protein consists of 82 amino acids, including a signal sequence of 35 amino acids and defensin domain of 47 amino acids. The protein sequence has a significant homology to γ2-zeathionin defensins. Since the specificity of defensin activity depends on the formation of disulphide bonds, the defensin protein was expressed in two systems (E. coli and yeast Pichia pastoris), which differ in their ability to catalyze disulphide bond formation. The purified protein severely inhibited Fusarium graminearum spore germination and hyphal growth when added to cultures at 1 or 10 µg ml⁻¹ and fungus viability was completely arrested at 100 µg ml⁻¹. Our results suggest that the overexpression of the defensin gene may be a useful strategy for producing cereal crops with resistance against Fusarium graminearum.

Brachypodium distachyon: A model plant system for studies in Fusarium graminearum pathology. C. Nasmith and R. Subramaniam, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-food Canada. 960 Carling Avenue, Ottawa Ontario, K1A 0C6.

Gibberella zeae (Schw.) Petch (Fusarium graminearum Schwabe) is the causal agent of wheat head blight. Difficulties associated with wheat genetics have compelled researchers to find appropriate plant model systems to study F. graminearum pathology. While Arabidopsis thaliana (L.) Heyn. has proven to be an invaluable tool to study plant-microbe interaction, it is a non-host to F. graminearum, making it difficult to effectively and efficiently study this fungal system. The grass species Brachypodium distachyon (L.) Beauv. has useful study features including a recently sequenced genome, reduced ploidy and genome size, as well as a much closer evolutionary relationship to wheat and other cereal crop species than A. thaliana. This has created great interest in B. distachyon as an alternative plant model system for plant-microbe studies. Previously untested for Fusarium infection, we have performed assays on B. distachyon resulting in the first documented accumulation of 15-ADON. Subsequent assays were performed that allowed separation of F. graminearum strains based on visible infection symptoms over time as well as by trypan blue staining. Further research will no doubt confirm that B. distachyon will be an indispensable tool required for Fusarium graminearum pathology studies.

To gain a better understanding of the difference in response to *Fusarium graminearum* infection between susceptible and resistant varieties of wheat, gene expression profiling is being performed using the Affymetrix wheat genome array on head tissues sampled up to 6 days after inoculation. All profiles are being compiled into a database using Acuity. We have compared the RNA profiles of four groups of wheat plants: 1) the spring wheat varieties Roblin (very susceptible), Wuhan 1 and NuyBay (both resistant, from Chinese and Japanese sources of resistance, respectively); 2) the spring wheat Chinese Spring (susceptible) and the introgression lines 7E and 7ES (both resistant, containing the chromosome 7 from *Thinopyrum elongatum* into Chinese Spring background); 3) the winter wheat Augusta (susceptible) and FH B148 (resistant, derived from Frontana, a Brazilian source of resistance); 4) near isogenic lines (derived from a cross between Wuhan 1 and NuyBay) that are segregating for the single FHB resistance QTL 2DL. Many genes (eg. in phenylpropanoid pathways) are induced by Fusarium in both susceptible and resistant varieties. Other genes respond differentially between resistant and susceptible varieties, including genes from hormone signalling pathways. The gene expression profiles for genes in the salicylic acid, jasmonic acid, ethanol and gibberellin signalling pathways will be summarised for the 4 group of plants studied.

Phosphopeptide analysis using proteomics: a basis for identifying signalling pathways active during DON synthesis in *Fusarium graminearum*. C. Rampitsch, G. Subramaniam, N.V. Bykova, S. Djuric-Ciganovic. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB R3T 2M9; (G.S.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa ON.

*Fusarium graminearum* produces well-characterized mycotoxins, principally deoxynivalenol, DON, and its derivatives which are toxic to humans and livestock. Although mycotoxin synthesis is well understood, molecular mechanisms regulating their production are not. This study examines the potential role of protein phosphorylation in regulating the onset of DON synthesis in nitrogen-starved *F. graminearum* grown *in vitro*. Multidimensional peptide separation and analysis (GeLCMS) and two-dimensional gel electrophoresis (2DE) were used to probe the phosphoproteome of *F. graminearum*. In the first biological replicate 53 unique phosphopeptides were identified from 3 samples (11 gel slices each) from *F. graminearum* grown on nitrogen-poor media at t = 0, 6h and 12h. These were from proteins involved in the regulation of protein synthesis, general metabolic enzymes, biosynthetic enzymes and proteins of unknown function. The second biological replicate is underway. By 2D electrophoresis, a further 34 putative phosphoproteins were identified, however, phosphorylation sites could only be assigned.
to 6 of these. The biological role of some of these proteins in the regulation of DON synthesis will be assessed in vivo by producing *F. graminearum* mutants and measuring both their virulence and ability to produce DON.

**Molecular approaches toward *Fusarium graminearum* resistance in corn (Zea mays L.)** Y. Reinprecht, X. Wu, E. Dasilva, S. Luk-Labey, C. J. Martin, and K. P. Pauls. *Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada.*

*Fusarium graminearum* causes gibberella ear rot in corn. The incidence and severity of the disease is strongly influenced by environmental factors and can reach epidemic levels. The infection can result in severe reduction in crop quality because of contamination of the grain with trichotheccene mycotoxins (deoxynivalenol, DON). The most feasible control is to use resistant hybrids. Unfortunately, most commercial corn hybrids have little or no resistance to infection by *Fusarium graminearum*. In a previous study, we identified several QTL for resistance to gibberella ear rot in corn. The focus of this work was to identify genes underlying the previously identified QTLs for resistance to gibberella ear rot in corn. Approximately 400 sequence-specific PCR primers were designed for ESTs for Fusarium infected corn, markers associated with FHB in wheat, ESTs that have been mapped to the Fusarium QTL regions in other studies, and likely candidate genes (ferulic acid synthesis, RPL3, and G protein genes) and screened with the parental DNA (CO387, resistant and CG62, susceptible). To date, 200 polymorphic markers have been scored in the mapping population. Some of the new markers cosegregate with previous Fusarium resistance QTLs. The identified resistance genes will be converted to markers that can easily be scored to allow rapid introgression of gibberella ear rot resistance into elite germplasm.


The overall goal is to develop Fusarium head blight (FHB) resistant wheat germplasm to minimize mycotoxin contamination of kernels. This project is part of synergistic cooperation with Agriculture and Agri-Food Canada, the University of Manitoba and the University of Guelph. The German subproject is structured into three modules.
Module 1: Molecular characterisation of resistance sources adapted to Central Europe
The focus is to identify and exploit genomic regions that confer FHB resistance in European winter wheats using biparental mapping populations, genome wide association mapping and validation of known resistance QTL.

Module 2: Introgression breeding of resistances from exotic sources
Aims are to examine the potential linkage drag for agronomic traits when introgressing multiple non-adapted spring wheat resistance QTL into elite winter wheat and to estimate the effect of these QTL on fitness of *Fusarium graminearum* populations. Additionally, characterization of new resistance donors in genetic resources (*Triticum* spp.) and introgression of these sources into adapted wheat will be performed.

Module 3: Functional genomics.
Expression profiling of Fusarium-attacked wheat spikes with respect to absence/presence of important resistance alleles, virus induced gene silencing (VIGS) and targeting induced local lesions in genomes (TILLING) are employed to discriminate between different mechanisms of quantitative resistance to FHB at the molecular level.

### Haplotype analysis of Fusarium resistance QTL in common and durum wheat from North America and Europe

L. Tamburic-Ilinic, D. Somers, A. Brulé-Babel and G. Fedak  
*Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, Ontario, N0P 2C0; (D. S.) Cereal Research Centre, AAFC, 195 Dafoe Road, Winnipeg, Manitoba, R3T 2M9; (A.B.) Dept. of Plant Science, 222 Agriculture Building, 66 Dafoe Road, University of Manitoba, Winnipeg, Manitoba, R3T 2N2; (G. F.) Eastern Cereals and Oilseeds Research Centre, AAFC, Central Experimental Farm, Ottawa, Ontario K1A 0C6*

Fusarium head blight (FHB) is an important disease of wheat. *Fusarium graminearum* (Schwabe) is the predominant species that causes FHB and produces the mycotoxin deoxynivalenol (DON) in the grain. The most practical way to control FHB is through the development of resistant cultivars. However, breeding for FHB resistance and low DON content in grain has been difficult because various types of resistance to FHB in wheat are quantitatively inherited. We have initiated studies to identify sources of resistance with which to bolster the known FHB resistance. Winter wheats from North America, France, Hungary, Czechoslovakia and Netherlands were haplotyped with microsatellite markers linked to FHB resistance quantitative trait loci (QTLs) on chromosomes 2D, 3A, 3B, 5A and 6B. The diagnostic band sizes for markers associated with QTLs for FHB resistance in winter wheat from North America and Europe will be reported. Parents with unique FHB resistance QTLs were identified, and strategies for pyramiding the various sources of resistance will be discussed.
Genome-Wide Linkage Disequilibrium Analysis and Association Study for Agronomic Traits in Canadian Barley. L.Y. Zhang, S. Marchand, and F. J. Belzile. Département de phytologie, Pavillon Marchand, Université Laval, Québec QC G1A 0A6 Canada.

Linkage disequilibrium (LD) mapping could be a valuable approach for identifying QTLs in barley. A collection of 188 lines representative of barley cultivated in Canada was genotyped with >1,000 DArT markers. The population was clearly defined into two groups based on the number of rows. LD was estimated using the squared allele-frequency correlation ($r^2$). On average, 22.4% of all marker pairs showed significant LD ($P<0.001$). There was a clear relationship between genetic distance and LD and the average $r^2$ value dropped below 0.2 for markers located 3-5 cM apart. Using a subset of 65 elite cultivars for which historical data were available, we measured marker-trait associations for plant height and test weight. Sixteen QTLs for plant height ($R^2$ between 0.0346 and 0.1467) and 22 QTLs for test weight ($R^2$ between 0.0506 and 0.1477) were identified. These were located on all of the chromosomes except 4H. Of the 16 QTLs for height, 7 mapped to a location where previous studies had reported height QTLs and of the 22 QTLs for test weight, 12 co-localized with previously reported test weight QTLs. In many instances where no such co-localization was found, poor marker coverage in the conventional QTL mapping populations was noted. Our results suggest that association mapping represents an attractive alternative to classical QTL methods.

Epidemiology and Disease Management/ Epidémiologie et Contrôle

Black barley as a means of minimizing the level of Fusarium head blight and deoxynivalenol contamination. T.M. Choo¹, B.J. Vigier¹, M. Savard¹, J.M. Yang², and J.M. Wang². ¹Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada; ²Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China.

Landraces of black barley are grown in many parts of the world, particularly in Syria and Tibet. Black barley contains higher protein, higher fibre, and higher vitamins than yellow barley. Black barley can be used for beer and breakfast cereals. We conducted a study to compare the level of FHB and deoxynivalenol (DON) between black and yellow barley. Tests were carried out at Harrington, P.E.I, Ottawa, ON and Hangzhou, China in 2005-2006. Results show black lines were heading later, were taller, and had lower FHB incidence, and lower DON accumulation than yellow lines. The differences between black and yellow barley were consistent over the two crosses of AC Klinck/CH9403-2 and AC Legend/CH9403-2 made at Charlottetown during the winter of 2000-2001. Both AC Klinck and AC Legend are yellow, six-row barley cultivars for Eastern Canada, while CH9403-2 is a black, six-row advanced breeding line. These results suggest that replacing yellow barley with black barley can reduce FHB incidence by 27-64% and
DON concentration by 15-51%. Additional testing for FHB and DON accumulation is underway. Yield and other agronomic traits of these black barley lines are also under investigation.

**Prediction models for fusarium head blight disease index and deoxynivalenol accumulation based on fusarium airborne inoculum level on wheat heads, cropping practices and weather conditions.** X.W. Guo, W.G.D. Fernando, H. Sapirstein, P. Bullock, J. Gilbert, and T. Nowicki, *Department of Plant Science; (H.S.) Department of Food Science; and (P.B.) Department of Soil Science, University of Manitoba; R3T 2N2; (J.G.) Agriculture and Agri-Food Canada; R3T 2M9; and (T.N.) Canadian Grain Commission; R3C 3G8, Winnipeg, Manitoba. Canada

The study was conducted in Manitoba, Canada, and consisted of 14 fields in 2003, 17 fields in 2004, and 22 fields in 2005. Two spring wheat cultivars Superb (moderately susceptible to FHB disease) and AC Barrie (intermediate in resistance to FHB disease) were sown. Two types of prediction models for FHB disease index and DON accumulation were developed on the basis of the number of *F. graminearum* / *Gibberella zeae* spores present on single wheat heads, cropping practices, and weather conditions. Type I models were developed using actual spore number, and prediction accuracy ranged from 87.9 % to 89.6 % for the disease index and from 77.9 % to 95.3 % for toxin level. Type II models were developed based on predicted spore number using a regression model, and prediction accuracy ranged from 64.3 % to 89.1 % for the disease index and from 76.6 % to 77.7 % for toxin level. The poster will discuss how these models will help wheat producers reduce FHB infection through management practices and assist the Canadian Wheat Board to reduce the risk of DON contamination in grain shipments.

**Concurrent selection for soil- and food-borne microbial suppression of *Fusarium graminearum*, Fusarium head blight and deoxynivalenol (DON) in wheat.** Jianwei He1,2, Greg J. Boland1, Ting Zhou* 2. 1: Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1, Canada; 2: Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, ON, N1G 5C9, Canada zhout@agr.gc.ca

*Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) causes Fusarium head blight (FHB) and produces deoxynivalenol (DON) in wheat (*Triticum aestivum* L.). In this research, concurrent screening methods were used to progressively select soil and food microorganisms for the ability to suppress *F. graminearum* and FHB in wheat. More than 250 microbial isolates were assessed using up to five *in vitro* assays including: a co-culture and dual-culture assay, an indirect impedance assay, a wheat floret assay, and assays assessing DON production on whole wheat flour and wheat florets, respectively. Ten isolates were chosen for subsequent evaluations using an *in vivo* greenhouse evaluation. *Paenibacillus polymyxa* Prazmowski W1-14-3 and *P. polymyxa*
C1-8-b gave the highest inhibition of *F. graminearum* and reduction of DON production in the greenhouse evaluation. Compared with a control treatment, these bacteria reduced disease severity by 58% and 59%, *F. graminearum* colonization of wheat heads by 63% and 68%, DON production by 91 and 92%, and increased 100-kernel weights by 88 and 86%, respectively. Concurrent selection of soil- and food-borne microorganisms for activity in reducing *F. graminearum*, FHB, and DON has resulted in promising antagonists that may possess multiple modes of action and the ability to colonize wheat heads in controlled environments. Field evaluation may further demonstrate the efficacy of these isolates as biocontrol agents.

*Keywords: Fusarium graminearum*, deoxynivalenol (DON), Fusarium head blight, soil, food microorganism, *Paenibacillus polymyxa*

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**Ultra-structural study of the effect of Trichoderma harzianum on the perithecial development of Gibberella zeae.** S. Inch and J. Gilbert. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg Manitoba, Canada, R3T 2M9.

In Manitoba, the principal pathogen associated with fusarium head blight of wheat is *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe). Perithecia form on crop residues and produce ascospores, the primary source of inoculum. A disruption in this stage in the life cycle may restrict inoculum development. We used scanning and transmission electron microscopy (SEM, TEM) to investigate the effects of *Trichoderma harzianum* (Rifai), a known biocontrol agent, on perithecial development of *G. zeae*. Petri dishes containing wheat straw agar were co-inoculated with spore suspensions of *G. zeae* and *T. harzianum*. Perithecia examined after 8, 10, and 28 days with the SEM from straw treated with *T. harzianum* showed abnormal development. The perithecia and initials were collapsed and closely associated with the spores and mycelium of *T. harzianum*. At 28 days after inoculation, the surface of the perithecia appeared smooth and lacked the large spherical cells associated with the outer wall seen in untreated samples. Cellular differences were also observed when viewed with the TEM. After 13 days, the cells of the outer wall of the perithecia from straw treated with *T. harzianum* had numerous large vacuoles and lacked the uniform cellular distribution seen in the untreated samples.

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**Variation of aggressiveness of German and Canadian isolates and isolate mixtures of Fusarium graminearum on highly resistant and susceptible spring wheat.** C. Knopf, and T. Miedaner. University of Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, D-70599 Stuttgart, Germany.

Effective quantitative trait loci (QTL) were introgressed intro wheat to combat *Fusarium* head blight (FHB). It is, however, not clear whether the fungal populations will adapt unspecifically to higher resistance levels in wheat. The effect of host resistance on *F.*
*graminearum* was tested with German and Canadian isolates and mixtures of German isolates on spring wheat entries with none to two resistance-QTL alleles in the field. Weather during inoculation favoured FHB infections resulting in mean FHB ratings ranging from 3.9 to 24%. All isolates could visibly infect also the highly resistant wheat genotype although mean FHB rating on this genotype was low ranging from 1.4 to 6.8%.

Single isolates and mixtures as well varied significantly (P<0.01) in their aggressiveness leading to high heritabilities (h² > 0.9) in both experiments. Host genotypes also were significantly different (P<0.01) and reacted in the expected order from the most resistant to the entry without QTLs being most susceptible. In both experiments, no significant interaction between isolates and wheat genotypes occurred although high resistance and aggressiveness levels could be realized. Hereafter we will examine by molecular markers whether the isolates within the mixtures differ in their relative abundance and whether differences are due to the resistance of host genotypes.

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**DON Content in Barley: Effect of Harvesting Date and Cultivar.** J. Lajeunesse, D. Pageau, and M.E. Savard. Research Farm, Agriculture and Agri-Food Canada, 1468 Saint-Cyrille Street, Normandin QC G8M 4K3; (M.E.S.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa ON K1A 0C6, Canada.

In Northern Quebec, Canada (Saguenay-Lac-Saint-Jean area), barley production is very important but Fusarium head blight (FHB) has become a major problem in this region. From 2003 to 2004, a trial was conducted at the Research Farm of Agriculture and Agri-Food Canada in Normandin to determine the effect of 3 harvesting dates (D1: maturity; D2: 5 days after maturity and D3: 10 days after maturity) and 3 cultivars (AC Klinck, Brucefield and Viviane) on deoxynivalenol (DON) content in barley. Harvesting date had no significant effect on DON content from 2003 to 2005. In 2006, at D3, yields were reduced by 12% and 9 % compared to D1 and D2 respectively. This could be explained by higher grain shattering when harvest was delayed. In 2003, 2004 and 2006, DON content of cultivar AC Klinck was significantly lower than that of cultivar Viviane and Brucefield. However, in 2005, DON content was the lowest with the cultivar Viviane compared to Brucefield. The interaction Harvesting date X Cultivar on DON content in barley was not significant.

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**Investigation about potential links between orange wheat blossom midge damage, Fusarium diseased kernels, and DON in wheat.** F. Langevin, A. Comeau and M. Savard. Soils and Crops Research and Development Centre, Agriculture and Agri-Food Canada, 2560 Hochelaga, Québec, QC, G1V2J3; and (M.S.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, K1A0C6.

The orange wheat blossom midge (*Sitodiplosis mosellana*) causes direct damage to bread wheat in Quebec, averaging above 6% according to estimates. Published results showed
that in controlled conditions, the insect transmits some *Fusarium graminearum*. Besides this, the hot humid climate conditions that favor Fusarium head blight (FHB) favor the midge as well. This prompted an investigation of the possible relationship between midge damage and Fusarium diseased kernels (FDK) in a number of seed lots. Results from yield trials (2002), and from FHB resistance trials (2005, 2006, and 2007) showed that there was roughly 3 times more FDK or DON in grains that had midge damage symptoms (compared to sound-looking grains). For example, in 2002 yield trials, the DON levels were 1.4 ppm for midge damaged grains, against 0.5 ppm for perfectly sound looking grains, which were the majority.

Considering evidence that midge adults (and/or larvae) can spread FHB, and the fact that midge damaged grains might contain more DON, there could be a reason to pay more attention to the midge resistance in breeding against FHB. The existence of links between midge, FHB, FDK and DON remains a hypothesis that could not be rejected. The detailed nature of the links is still not fully defined.

**How nitrogen fertilization and preceding crop affect deoxynivalenol content in barley.** D. Pageau, J. Lafond, J. Lajeunesse and M.E. Savard. *Research Farm, Agriculture and Agri-Food Canada, 1468 St-Cyrille street, Normandin QC G8M 4K3; (M.E.S.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, ON K1A 0C6.*

Since 2001, in the Saguenay-Lac-Saint-Jean area (Quebec), Fusarium head blight has become a major problem for many barley producers. The effect of four preceding crops (barley, pea, red clover and soybean) and four nitrogen addition rates (0, 40, 80 and 120 kg ha\(^{-1}\)) on deoxynivalenol (DON) content in barley were evaluated from 2002 to 2005. In 2002, 2003 and 2005, DON content was significantly lower when barley followed soybean, red clover or pea compared to barley following barley. In 2004, DON content was low for all the treatments and the previous crop had no significant effect on DON content in barley. The effect of nitrogen fertilization on DON content was not significant during the four years.

**Infection of crowns of wheat and barley by seed-borne inoculum of *Fusarium graminearum*.** S. Pouleur, L. Couture, A. Comeau, and R.M. Clear. *Research Centre, Agriculture and Agri-Food Canada, 2560 Hochelaga Blvd., Québec, QC, G1V 2J3, Canada; and (R.M.C.) Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, MB, R3C 3G8, Canada.*

A high proportion of seeds carrying Fg (*Fusarium graminearum* Schwabe) may be found in certified cereal seed lots. To assess the extent such inoculum can infect plants in the field, we compared the infection level of crowns grown from seeds of wheat and barley with high (0 = 48%) and low (0 = 10%) contamination levels, treated or not with a
Fungicide. Field tests were conducted at two sites in Quebec in 2002 and 2003. A few weeks post-harvest, culms were collected and dried, crowns excised and contamination by Fg (%) determined by plating pieces of crown onto peptone-PCNB agar. In 2002, crown infection was significantly higher (more than double) with the highly contaminated seeds although the level of infection varied with site and crop. Seed treatment had no effect. In 2003, trends were similar, but recovery of Fg was very low, perhaps because of fungal death during drying. These results confirm that seeds contaminated with Fg could increase the incidence of Fg in the crop. Accordingly, long distance dispersal of more virulent strains and the build-up of genetic diversity of the pathogen can be promoted. The inability of seed treatment to control transmission of Fg to the crown supports the use of less contaminated seeds for grain production.

Application of bacterial antagonists for controlling of wheat Fusarium head blight (FHB), using single and mixture strains on different cultivars. M. Javad Soleimani¹*, Hamid Rohani² and Mahsa Alimi¹. ¹Dept. of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, IRAN; ²Dept. of Plant Sciences, Faculty of Agriculture, Ferdosi University, Mashhad, IRAN; Corresponding Author e-mail: agrms@basu.ac.ir*

Fusarium head blight (FHB) incited by Fusarium graminearum Schwabe, is a devastating disease that causes extensive yield and quality losses to wheat (Triticum aestivum L.) throughout the world. One strategy to control FHB is the use of antagonistic bacteria. In order to assess the potential of phyllospheric microorganisms in biological control of such foliage diseases, in this study one hundred ninety isolates of antagonistic bacteria including Pseudomonas, Erwinia and Bacillus spp. from phyllosphere of healthy and infected wheat, were collected. Among them, by using the dual culture method, only eight isolates with the most antagonistic ability against the growth of pathogenic fungal species (F. graminearum) were selected and purified. According to the results of biochemical and physiological tests, they were identified as three biovars of Pseudomonas fluorescens, an isolate of Erwinia herbicola and some species of Bacillus like B. subtilis, and B. cereus. Production of antifungal substances and volatile metabolites, and secretion of lytic enzymes such as protease and cellulase as the inhibitory mechanisms in vitro were evaluated. Furthermore, in greenhouse conditions the effects of antagonistic bacteria on disease severity and incidence caused by F. graminearum, by the application of bacteria were studied. Statistical analysis of data indicated that, treating wheat spikes with some of the antagonistic bacteria, not only reduced the disease severity and incidence, compared to the control, but also showed a positive influence on growth and yield of wheat cultivars.

Key words: Biological control, Fusarium head blight (FHB), Fusarium graminearum, Pseudomonas fluorescens, Erwinia herbicola, Bacillus subtilis
**Fusarium species and mycotoxins of oat in Ontario, Canada.** L. Tamburic-Ilinic

Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, Ontario, N0P 2C0

*Fusarium graminearum* (Schwabe) [teleomorph: *Gibberella zeae* Schw. (Petch)] is a predominant species of *Fusarium* that is pathogenic to cereals in Ontario and produces the mycotoxin deoxynivalenol (DON) in grain. The objectives of this study were to determine the *Fusarium* spp. and concentrations of mycotoxins from commercial oat fields grown in the same area as wheat and barley in Ontario and to estimate FHB index and DON level across cultivars grown in the Ontario Performance Trial (OPT) after spray-inoculation with *F. graminearum*. Grain samples were retrieved from oat fields randomly selected across Ontario from 2005 to 2007. The top three *Fusarium* species were *F. graminearum*, *F. sporotrichioides* and *F. poae*. The highest concentrations of measured DON, HT-2, and T-2 were 0.3 ppm, 0.5 ppm and 0.2 ppm, respectively. Under natural infections, differences in DON content were observed among farm field locations, but not among cultivars. However, differences in FHB index and DON level amongst the cultivars grown in the OPT were identified after inoculation. In 2007, FHB index and DON level ranged from 3.5% to 37.0% and from 0.1 ppm to 1.1 ppm, respectively. This study supports the importance of monitoring *Fusarium* spp. and concentrations of mycotoxins in oat, especially during weather conditions that favor *Fusarium* infection and mycotoxin accumulation.

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**Are fusarium head blight survey data meaningful and reliable?** A. Tekauz.

Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB, Canada, R3T 2M9.

Surveys to document the prevalence of fusarium head blight (FHB) in cereal crops in Manitoba have done annually for many years. These are conducted prior to crop maturity to enable discrimination between healthy and diseased spike tissue and allow for estimation of disease incidence and severity. The results constitute an ‘early-warning system’ that FHB may be causing significant damage to crops. In addition, sampling of putatively affected spikes at this time for the presence and identity of *Fusarium* species provides verification of the disease, and allows for tracking of any changes in the relative proportion and composition of the causal species. FHB can also be assessed in the harvested grain, when levels of fusarium damaged kernels or FDK, the mycotoxin deoxynivalenol or DON, and *Fusarium* kernel infestation are determined to provide another set of empirical and comparative data. Are these two approaches to gauge FHB comparable and complementary? One field of each of barley, oat and spring wheat were surveyed in 2007 first at the soft dough stage, which is typical timing for farm field surveys, and again following harvest. There were interesting differences and similarities between the results obtained from the two survey methods. These need to be considered and understood, when such data are examined for their implication and relevance to both the primary and value-added sectors of the industry.
Genetic changes in the Canadian *Fusarium graminearum* population and their effect on pathogenicity, toxin production, fungicide sensitivity, and disease spread. T.K. Turkington, R. Clear, J. Gilbert, T. Nowicki, K. O’Donnell, A.P. Rooney, A. Tekauz, and T.J. Ward. Lacombe Research Centre, Agriculture and Agri-Food Canada, Lacombe, AB, T4L 1W1; (J.G., A.T.) Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB R3T 2M9; (R.C., T.N.) Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg MB R3C 3G8, Canada; (K.O., T.W., A.P.R.) United States Department of Agriculture (USDA), Peoria, IL, USA.

Until recently, isolates of *Fusarium graminearum* Schwabe of the ‘15ADON’ chemotype were the major cause of fusarium head blight (FHB) in North America. However, molecular surveillance has revealed that isolates with a 3ADON chemotype are now displacing 15ADON isolates, in Canada and elsewhere. This could have significant implications for producers because 3ADON isolates accumulate significantly more deoxynivalenol (DON) and consequently may present more challenges for marketing of Fusarium infected grains. The bases for these changes in *Fusarium* composition, and their implication for disease control efforts, are unclear. The apparent rapid and significant shift in chemotype in the pathogen suggests that Canadian *F. graminearum* isolates with a 3ADON chemotype have a selective advantage. The fact that isolates with a novel, more toxic trichothecene profile are proliferating has significant implications for cereal production and food safety. A new research project funded by the Western Grains Research Foundation will elucidate the distribution and movement of highly toxigenic 3ADON isolates, assess the basis for their rapid spread and apparent selective advantage, and evaluate implications for current FHB management efforts. This information will also be critical to the formulation of informed regulatory policy, potential fungicide resistance strategies, and the development of cereal cultivars with broad-based resistance to trichothecene-producing fusaria.

Effect of harvesting time on grain contamination with *Fusarium* species and deoxynivalenol in barley in Ontario. A.G. Xue, A.M. Manceur, J. Rowsell, K.M. Ho, T.M. Choo, and Y. Chen. Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, K.W. Neathy Building, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada; (A.M.M.) Faculty of Forestry, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; (J.R.) New Liskeard Agricultural Research Station, Box 6007, 340 Armstrong St., New Liskeard, ON P0J 1P0, Canada.

The effect of harvest time on incidence of seedborne *Fusarium* spp. and deoxynivalenol (DON) concentration in barley was studied using cultivars AC Vision, Brucefield, and OAC Baxter at three locations (Emo, New Liskeard, Ottawa) in northern and eastern Ontario each year in 2004 and 2005. The seedborne *Fusarium* spp. were dominated by *Fusarium equiseti* and *F. sporotrichioides*, recovered from 4.3% and 3.2% of the kernels and represented 28.9% and 28.7% of the pathogen population, respectively. *Fusarium poae*, *F. graminearum*, and *F. avenaceum* were recovered from less than 2% of the kernels and represented 7.2%, 6.1%, and 1.4% of the population, respectively. Other species including *F. acuminatum*, *F. culmorum* and *F. semitectum* were rarely found and
collectively represented less than 1% of the population. The incidences of total *Fusarium* spp. increased from 4.2 to 9.4% while DON concentration followed no clear pattern with the delayed harvesting time. Of the frequently recovered species, only *F. sporotrichioides* and *F. avenaceum* increased with the delayed harvest. The DON concentration in the harvested grain of this study ranged from 0.1 to 0.5 ppm with the mean of 0.3 ppm, which is below the Canadian tolerance level of 1.0 ppm for swine feed, suggesting that fungicide application to control fusarium head blight and reduce DON content in grain may not be necessary in non-epidemic years.

**Biological control of fusarium head blight of wheat with *Clonostachys rosea* strain ACM941.** A.G. Xue, X.L. Tian, H.D. Voldeng, M.E. Savard, and Y. Chen. *Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada; (X.L.T.) Department of Environmental Biology, University of Guelph, Guelph, ON N1G 2W1, Canada.

A strain of *Clonostachys rosea*, ACM941 (ATCC #74447) was evaluated for its antibiosis to *G. zeae in vitro* and for controlling of fusarium head blight (FHB) under both greenhouse and field conditions, in comparison to the registered fungicide Folicur (tebuconazole). ACM941 reduced the mycelial growth of the pathogen by 53% in dual culture and completely suppressed the macroconidium germination of *G. zeae* in co-culture for 6 hours. ACM941 reduced the peritheciun production by more than 99% in leaf disc assay, 23-57% on debris, and 36-70% on infested kernels. When sprayed onto wheat heads prior to inoculation with *G. zeae*, ACM941 significantly reduced infected spikelets (IS) by 58-71% and fusarium damaged kernels (FDK) by 59-73% compared to the untreated disease control. Under the simulated natural epidemic conditions during 2005-2007, ACM941 reduced IS by 44-51%, FDK by 33-68%, and deoxynivalenol (DON) in grains by 10-28%. ACM941 was similar to Folicur in reducing the mycelial growth, spore germination, and peritheciun production of *G. zeae*, but was less effective than Folicur in reducing IS, FDK, and DON in the field. Results of this research suggest that ACM941 is an effective antagonist against *G. zeae* and may be used as an alternative of chemical fungicides in an integrated FHB management program.
Quality and End-Use Safety/ Qualité et sécurité

Effect of Fusarium Head Blight on Hard White Wheat Quality
D. Fenn, O. Lukow, D. Abramson, G. Humphreys, J. Gilbert, D. Brown, R. DePauw and N. White. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada. Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada.

Quality characteristics of Fusarium Head Blight (FHB)-infected wheat of the Canada Western Hard White Spring class, variety Snowbird, were studied. The distribution of deoxynivalenol (DON) among mill streams and the effect of pearling, straight-grade flour milling, bread and noodle-making on DON concentration were investigated. An enzyme immunoassay was used for determining DON levels. Three Snowbird samples with different DON levels were milled into eight flour fractions using a Buhler experimental mill. On average, the shorts and bran had the highest DON concentrations, whereas the reduction flours had the lowest DON levels. Milling of twelve samples into straight-grade flour resulted in an average DON level reduction of 22%. An additional average of 16% and 51% of the wheat DON level were reduced by further processing into bread and cooked kansui noodle, respectively. Ten FHB-infected wheat samples were also fed through an abrasive bench top pearler at seven different time intervals ranging from 25 sec to 225 sec. Kernel DON concentration was reduced as pearling time increased.

Effects of electron beam irradiation on deoxynivalenol in distillers dried grain and solubles and in production intermediates. T. Stepanik and D. Kost. Acsion Industries Incorporated, 402-Ara Mooradian Road, Whiteshell Laboratories, Pinawa, MB Canada, R0E 1L0; T.W. Nowicki and D. Gaba, Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, MB Canada R3C 3G8

The marketability of the byproducts of ethanol production is critical to the economic health of this industry. Fusarium head blight (FHB) infection of cereals and the resulting presence of Fusarium mycotoxins such as deoxynivalenol (DON) in distillers dried grain and solubles (DDGS) is a major concern. This study investigated the feasibility of using irradiation to reduce the retention of DON in DDGS. DON contaminated wheat and distillers dried grain and solubles (DDGS) were irradiated to doses ranging from 2 to 55.8 kGy using an electron accelerator. Samples of wet distillers grain, distillers solubles and stillage obtained during production of DDGS were also irradiated. Samples were analyzed for Fusarium trichothecene mycotoxins by gas chromatography-mass spectrometry (GC-MS). The three production intermediates showed dose-dependent reductions in their DON contents ranging from 47.5 to 75.5 % at the highest doses. Electron beam (EB) treatment produced a 17.6 % reduction in the DON level of wheat at the highest dose used, but had no effect on DON in DDGS. These results indicate that EB treatment may provide a method for reducing DON levels in DDGS on an industrial scale.
# List of Registrants

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<td>Dale Alderson</td>
<td>Paterson Global Foods Inc</td>
<td>Winnipeg MB, R2E 0K7</td>
<td>204-956-2090</td>
<td><a href="mailto:dalderson@patersongrain.com">dalderson@patersongrain.com</a></td>
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<td>Winnipeg, MB, R3X 2J2</td>
<td>204-223-0630</td>
<td><a href="mailto:kurt.anada@syngenta.com">kurt.anada@syngenta.com</a></td>
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<tr>
<td>Erin Armstrong</td>
<td>Brewing &amp; Malting Barley Research Institute</td>
<td>Winnipeg MB, R3B 2L6</td>
<td>204-927-1401</td>
<td><a href="mailto:earmstrong@bmbri.ca">earmstrong@bmbri.ca</a></td>
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<td>Guy Ash</td>
<td>Canadian Wheat Board</td>
<td>Winnipeg MB, R3C 2P5</td>
<td>204-984-6820</td>
<td><a href="mailto:guy_ash@cwb.ca">guy_ash@cwb.ca</a></td>
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<td>Eva Beimcik</td>
<td>Cereal Research Centre</td>
<td>Montreal QC, H3C 3J7</td>
<td>514-343-6273</td>
<td><a href="mailto:ebeimcik@agr.gc.ca">ebeimcik@agr.gc.ca</a></td>
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<td>Francois Belzile</td>
<td>Laval University</td>
<td>Quebec QC, G1V 0A6</td>
<td>418-656-2131</td>
<td><a href="mailto:fbelzile@rsvs.ulaval.ca">fbelzile@rsvs.ulaval.ca</a></td>
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<td>Meconnen Beyeen</td>
<td>Cereal Research Centre</td>
<td>Winnipeg MB, R3T 2M9</td>
<td>204-984-0795</td>
<td><a href="mailto:mbeyene@agr.gc.ca">mbeyene@agr.gc.ca</a></td>
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<td>204-983-4604</td>
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<td>Leslie Bezte</td>
<td>Cereal Research Centre</td>
<td>Winnipeg MB, R3T 2M9</td>
<td>204-983-0936</td>
<td><a href="mailto:lbezte@agr.gc.ca">lbezte@agr.gc.ca</a></td>
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<tr>
<td>Jim Bole</td>
<td>FarmPure Inc.</td>
<td>Winnipeg MB, R3T 3S7</td>
<td>204-275-2259</td>
<td><a href="mailto:jboles@farmpure.com">jboles@farmpure.com</a></td>
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</tr>
</tbody>
</table>
Crosby Devitt
Ontario Wheat Producers' Marketing Board
Guelph ON N1G 5L3
519-767-6537
519-767-9713
crosby.devitt@ontariowheatboard.com

James Dexter
Canadian Grain Commission
Winnipeg MB R3C 3G8
204-983-5362
204-983-0724
jdexter@grainscanada.gc.ca

Ashley Dickson
Syngenta Crop Protection Canada Inc.
Plattville ON NOJ 1S0
519-696-2269
ashley.dickson@syngenta.com

Yves Dion
CEROM
Saint-Mathieu-de-Beloeil J3G 4S5
450-464-2715
450-464-8767
yves.dion@cerom.qc.ca

Slavica Djuric-Ciganovic
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-2498
204-983-4604
sciganovic@agr.gc.ca

Jim Downey
SeCan
Elstow SK S0K 1M0
306-257-3645
306-257-3647
jdowney@secan.com

Patrick Doyle
University of Guelph
Guelph ON N1G 4Z3
519-837-5314
519-823-0504
patrick.doyle@syngenta.com

Karen Dupchak
Manitoba Agriculture, Food + Rural Initiatives
Winnipeg MB R3T 5S6
204-954-7668
204-945-4327
karen.dupchak@gov.mb.ca

Erhard Ebmeyer
Lochow-Petkus GmbH
Bergen, Germany 29303
49-5051-4770
49-5051-447-22-142
leslie@pochow-petkus.de

Monica Eng
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-0936
204-983-4604
engm@agr.gc.ca

Mark Etienne
Hyland Seeds
Ailsa Craig ON N0M 1A0
519-232-4341
519-232-4345
metienne@hylandseeds.com

Francois Eudes
Lethbridge Research Centre
Lethbridge AB T1J 4B1
403-317-3338
403-382-3156
eudesf@agr.gc.ca

Duane Falk
University of Guelph
Guelph ON N1G 2W1
519-824-4120 x 53579
519-763-8933
dfalk@uoguelph.ca

Tao Fan
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-0476
204-983-4604
tfan@agr.gc.ca

George Fedak
ECORC
Ottawa ON K1A 0C6
613-759-1393
613-759-6559
fedakga@agr.gc.ca

Dilantha Fernando
University of Manitoba
Winnipeg MB R3T 2N2
204-474-6072
D_Fernando@umanitoba.ca
Nora Foroud
Lethbridge Research Centre
Lethbridge AB  T1J 4B1
403-317-2269
403-382-3156
foroudn@agr.gc.ca

Doug Fotheringham
Dow AgroSciences
Carman MB  R0G 0J0
204-750-1307
204-745-6338
dfotheringham@dow.com

Stephen Fox
Cereal Research Centre
Winnipeg MB  R3T 2M9
204-983-0573
204-983-4604
sfox@agr.gc.ca

Don Gaba
Canadian Grain Commission
Winnipeg MB  R3C 3G8
204-984-5704
204-983-0724
dgaba@grainscanada.gc.ca

Eugene Gawalko
Canadian Grain Commission
Winnipeg MB  R3C 3G8
204-983-8995
204-983-0724
mroscoe@grainscanada.gc.ca

David Gehl
Indian Head Research Farm
Indian Head  SK  S0G 2K0
306-695-5266
306-695-3445
gehl@agr.gc.ca

Jeannie Gilbert
Cereal Research Centre
Winnipeg MB  R3T 2M9
204-983-0891
204-983-4604
jgilbert@agr.gc.ca

Saber Golkari
Cereal Research Centre
Winnipeg MB  R3T 2M9
204-983-0487
204-983-4604
sgolkari@agr.gc.ca

Robert Graf
Lethbridge Research Centre
Lethbridge AB  T1J 4B1
403-317-2258
403-382-3156
grafr@agr.gc.ca

Mike Grenier
Canadian Wheat Board
Winnipeg MB  R3C 2P5
204-983-2996
204-984-1699
mike_grenier@cwb.ca

Tajinder Grewal
University of Saskatchewan
Saskatoon SK  S7N 5A8
306-966-8476
306-966-5015
tajinder.grewal@usask.ca

Steve Haber
Cereal Research Centre
Winnipeg MB  R3T 2M9
204-983-1467
204-983-4604
shaber@agr.gc.ca

Linda Harris
ECORC
Ottawa ON  K1A 0C6
613-759-1314
613-759-6566
harrislj@agr.gc.ca

Lorenz Hartl
Bavarian State Research Center
Freising, Germany  85354
49-8161-713814
49-8161-714085
lorenz.hartl@lff.bayern.de

Jian Wei He
Guelph Food Research Centre
Guelph ON  N1G 5C9
519-829-2400 x 3003
519-829-2600
heij@agr.gc.ca

Brian Hellegards
James Richardson Intl.
Howden MB  R5A 1K2
204-269-2722
204-269-1242
kelburn@jri.ca
Scott Henry
Bayer Crop Science
Winnipeg MB R3Y 1N4
204-989-5435
204-487-3648
scott.henry@bayercropscience.com

Peter Hicklenton
AAFC
Kentville NS B4N 1J5
902-679-5760
902-679-5344
hicklentonp@agr.gc.ca

Brenda Hoehn
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-0827
204-983-4604
hoehnb@agr.gc.ca

Charla Hollingsworth
University of Minnesota
Crookston MN 56716
218-281-8627
218-281-8603
holli030@umn.edu

Charlotte Hoorne
Intertek
Wi MB R3B 0P4
204-944-1887
204-942-0334
charlotte.hoorne@intertek.com

Jim House
University of Manitoba
Winnipeg MB R3T 2N2
204-474-9523
204-474-7628
j_house@umanitoba.ca

Tyler Huck
Syngenta Seeds Canada Inc
Morden MB R6M 1M8
204-822-5411
204-822-5459
tyler.huck@syngenta.com

Pierre Hucl
University of Saskatchewan
Saskatoon SK S7N 5A8
306-966-8667
306-966-5015
pierre.hucl@usask.ca

Gavin Humphreys
Cereal Research Centre
Winnipeg MB R3T 2M9
204-984-0123
204-983-4604
ghumphreys@agr.gc.ca

Todd Hyra
SeCan
Winnipeg MB R3P 1M9
204-489-9126
204-489-3615
thrya@secan.com

Sharon Inch
Cereal Research Centre
Winnipeg MB R3T 2M9
204-984-0223
204-983-4604
sinch@agr.gc.ca

Peter Johnson
Ontario Ministry of Agriculture
Stratford ON N5A 5T8
519-318-7769
519-273-5278
peter.johnson@ontario.ca

Rich Joy
Rahr Malting Canada
Alix AB T0C 0B0
403-747-2777
403-747-2068
rjoy@rahr.com

Ron Kaethler
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-5533
204-983-4604
rkaethler@agr.gc.ca

Andre Kalikililo
AAFC
Ottawa ON K1A 0C6
613-759-1845
613-759-6564
kalikililoa@agr.gc.ca

David Kaminski
Manitoba Agriculture, F&RI
Carman MB R0G 0J0
204-745-5656
204-745-5690
david.kaminski@gov.mb.ca
Pragya Kant
University of Guelph
Guelph ON N1G 2W1
519-824-4120
519-763-8933
pkant@uoguelph.ca

Bettina Kessel
KWS Saat AG Einbeck,
Lower Saxony, Germany 37574
49-5561-311-321
49-5561-311-337
b.kessel@kws.com

Lawrence Klusa
Canadian Wheat Board
Winnipeg MB R3C 2P5
204-983-4410
204-984-1699
lawrence_klusa@cwb.ca

Christiane Knopf
University of Hohenheim
Stuttgart, Germany 70599
49-711-459-23128
49-711-459-23841
knopf@lsa.uni-hohenheim.de

Uwe Kromer
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-5533
204-983-4604
ukromer@agr.gc.ca

Anastasia Kubinec
Canterra Seeds
Winnipeg MB R3T 1Y7
204-988-9760
204-487-7682
a.kubinec@canterra.com

Lanette Kuchenski
Western Grains Research Foundation
Saskatoon SK S7N 3R2
306-975-0060
306-972-0316
lkuchenski@westerngrains.com

Ajjamada Kushalappa
McGill University
Ste Anne de Bellevue QC H9X 3V9
514-398-7867
514-398-7897
ajjamada.kushalappa@mcgill.ca

Ted Labun
Syngenta Crop Protection Canada Inc.
Calgary AB T3B 5X9
403-863-9594
ted.labun@syngenta.com

Paul Laflamme
Alberta Agriculture & Food
Edmonton AB T6H 5T6
780-427-2166
780-427-1057
paul.laflamme@gov.ab.ca

Julie Lajeunesse
Research Farm
Normandin QC G8M 4K3
418-274-3378 x 239
418-274-3386
lajeunesseju@agr.gc.ca

Francois Langevin
AAFC
Quebec QC G1V 2J3
418-657-7985 x 5137
418-648-2402
langevinf@agr.gc.ca

Roger Larios
University of Manitoba
Winnipeg MB R3T 2N2
204-474-6104
204-474-7528
rlarios@ms.umanitoba.ca

Jamie Larsen
Hyland Seeds
Ailsa Craig ON N0M 1A0
519-232-4341
519-232-4345
jlarsen@uoguelph.ca

William Laskar
Pioneer Hybrid International

Bill Legge
Brandon Research Centre
Brandon MB R7A 5Y3
204-578-3600
204-728-3858
blegge@agr.gc.ca

Lorne Letkeman
Syngenta
Portage la Prairie MB R1N 3T1
204-871-2174
lorne.letkeman@syngenta.com
Victor Limay-Rios  
Guelph University  
Ridgetown ON N0P 2C0  
519-674-1567  
519-674-1555  
vlimayri@ridgetownc.uoguelph.ca

Gary Lombaert  
Health Canada  
Winnipeg MB R3J 3Y1  
204-984-2088  
204-983-5547  
gary_lombaert@hc-sc.gc.ca

Ali Malihipour  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-983-1463  
204-983-4604  
malihipoura@agr.gc.ca

Suzanne Marchand  
University Laval  
Quebec QC G1V 0A6  
418-656-2131 x 13102  
418-656-7176  
suzanne.marchand@plg.ulaval.ca

C. Joe Martin  
University of Guelph  
Guelph ON N1G 2W1  
519-824-4120 x 58180  
519-763-8933  
cmart07@uoguelph.ca

Richard Martin  
Crops & Livestock Research Centre  
Charlottetown PEI C1A 4N6  
902-566-6851  
902-566-6821  
martinba@agr.gc.ca

Peter Matthews  
ECORC  
Ottawa ON K1A 0C6  
613-759-1627  
613-759-5697  
matthewph@agr.gc.ca

Ana Matu  
Ontario Ministry of Agriculture  
Guelph ON N1G 4X2  
519-826-7907  
519-826-3233  
ana.matu@ontario.ca

Wayne Mauthe  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-983-2498  
204-983-4604  
wmauthe@agr.gc.ca

Brent McCallum  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-983-0771  
204-983-4604  
bmccallum@agr.gc.ca

Kevin McCallum  
AgriPro  
Morden MB R6M 1Y9  
204-822-5412  
204-822-5459  
kevin.mccallum@syngenta.com

John McGregor  
Manitoba Agriculture, F& RI  
Steinbach MB R5G 1N6  
204-371-1759  
204-326-4309  
John.McGregor@gov.mb.ca

Terry McIntee  
BioVision Seed Labs  
Edmonton AB T6B 3J4  
780-436-8822  
780-437-6875  
terrym@biovision.ca

Marcia McMullen  
Dept. of Plant Pathology  
North Dakota State University  
Fargo ND 58105  
701-231-7627  
701-231-7851  
marcia.mcmullen@ndsu.edu

Mohamed Mergoum  
North Dakota State University  
Fargo ND 58105-5051  
701-231-8478  
701-231-8474  
mohamed.mergoum@ndsu.edu

Thomas Miedaner  
Universitaet Hohenheim  
Stuttgart, Germany D-70593  
49-711-459-22690  
49-711-459-23841  
miedaner@uni-hohenheim.de
Shea Miller
AAFC
Ottawa ON K1A 0C6
613-759-1760
613-759-1701
millers@agr.gc.ca

Heather Milne
BioVision Seed Labs
Edmonton AB T6B 3J4
780-436-8822
780-437-6875
heatherm@biovision.ca

Debbie Miranda
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-0936
204-983-4604
dmiranda@agr.gc.ca

Jennifer Mitchell Fetch
Cereal Research Centre
Winnipeg MB R3T 2M9
204-983-1460
204-983-4604
jfetch@agr.gc.ca

Robin Morrall
Discovery Seed Labs Ltd
Saskatoon SK S7J 4M2
306-249-4484
306-249-4434
robin.morrall@usask.ca

Ricardo Carlos Moschini
Instituto de Clima y Agua CNIA INTA
Castelar Hurlingham,
Buenos Aires Argentina B1712WAA
54-11-4621-0125/1463
54-11-4621-5683
rmoschini@cnia.inta.gov.ar

Eric Mueller
Cereal Research Centre
Winnipeg MB R3T 2M9
204-984-0795
204-983-4604
emueller@agr.gc.ca

Charles Nasmith
AAFC
Ottawa ON K1A 0C6
613-759-7928
charles.nasmith@utoronto.ca

John Nelson
Neogen Corporation
Lansing MI 48912
517-372-9200
517-372-0108
tritter@neogen.com

Gary Neumann
Health Canada
Winnipeg MB R2J 3Y1
204-983-5490
204-983-5547
gary_neumann@hc-sc.gc.ca

Ian Nichols
Weather Innovations Inc
Chatham ON N7M 5J5
519-352-5334
519-352-7630
inichols@weatherinnovations.com

Sasanda Nilmalgoda
Cereal Research Centre
Winnipeg MB R3T 2M9
204-984-3505
204-983-4604
nilmalgodas@agr.gc.ca

Thomas Nowicki
Grain Research Laboratory
Winnipeg MB R3C 3G8
204-983-3345
204-983-0724
tnowicki@grainscanada.gc.ca

Henry Olechowski
Hyland Seeds
Blenheim ON N0P 1A0
519-676-8146
519-676-5674
holechowski@hylandseeds.com

Therese Ouellet
AAFC
Ottawa ON K1A 0C6
613-759-1658
613-759-1701
ouellettr@agr.gc.ca

Denis Pageau
Research Farm
Normandin QC G8M 4K3
418-274-3378
418-274-3386
pageaud@agr.gc.ca
Sylvie Rioux  
CÉROM  
Quebec QC  G1P 3W8  
418-528-7896  
418-644-6655  
sylvie.rioux@cerom.gc.ca

Mike Roscoe  
Canadian Grain Commission  
Winnipeg MB  R3C 3G8  
204-984-7457  
204-983-0724  
mroscoe@grainscanada.gc.ca

Brian Rosnagel  
Crop Development Centre  
Saskatoon SK  S7N 5A8  
306-966-4976  
306-966-5015  
brian.rossnagel@usask.ca

Mory Rugg  
AgriPro  
W. Fargo ND  58078  
701-298-0511  
mory.rugg@agripro.com

Don Salmon  
Alberta Agriculture & Food  
Lacombe AB  T4L 1W9  
403-782-8694  
403-782-5514  
donald.salmon@gov.ab.ca

Nana Sato  
Kitami Agricultural Experiment Station  
Tokoro-gun, Japan  099-1496  
81-157-47-3806  
81-157-47-2774  
s.nana@agri.pref.hokkaido.jp

Marc Savard  
AAFC  
Ottawa ON  K1A 0C6  
613-759-1683  
613-759-1701  
savardme@agr.gc.ca

Danny Saydak  
Canadian Grain Commission  
Winnipeg MB  R3C 3G8  
204-984-3108  
204-983-2751  
dsaydak@grainscanada.gc.ca

Art Schaffsma  
University of Guelph  
Ridgetown ON  N0P 2C0  
519-674-1505  
519-674-1515  
aschaafs@ridgetownc.uoguelph.ca

Michael Schmolke  
Technische Universitaet Muenchen  
Freising, Germany  85350  
49-8161-713488  
49-8161-714511  
michael.schmolke@wzw.tum.de

Joerg Schondelmaier  
Saaten-Union Resistenzlabor GmbH  
Leopoldshoehe, Germany  33818  
49-5208-9504-93  
49-5208-9504-94  
schondel@saaten-union-labor.de

Patrick Schweizer  
Leibniz-Institute of Plant Genetics  
Gatersleben, SA, Germany  D-06466  
49-39482-5660  
49-39482-5692  
schweiz@ipk-gatersleben.de

Steven Scofield  
USDA-ARS  
West Lafayette IN  47907  
765-494-3674  
765-496-2926  
scofield@purdue.edu

Peter Scott  
NB Dept Agriculture and Aquaculture  
Wicklow NB  E7L 3S4  
506-392-5100  
506-392-5089  
peter.scott@gnb.ca

Keith Seifert  
ECORC  
Ottawa ON  K1A 0C6  
613-759-1378  
613-759-1701  
seifertk@agr.gc.ca

Jyoti Shah  
University of North Texas  
Denton TX  76203  
940-565-3535  
940-565-4136  
shah@unt.edu
Dan Simard  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-984-0945  
204-983-4604  
simarredd@agr.gc.ca

Asheesh Singh  
SPARC  
Swift Current SK S9H 3X2  
306-778-7256  
306-778-3188  
singhak@agr.gc.ca

Jas Singh  
ECORC  
Ottawa ON K1A 0C6  
613-759-1662  
613-759-1701  
singhja@agr.gc.ca

Linnea Skoglund  
Busch Agricultural Resources  
Fort Collins CO 80524  
970-482-2332  
970-472-2334  
linnea.skoglund@anheuser-busch.com

Kevin Smith  
University of Minnesota  
St. Paul MN 55117  
612-624-1211  
612-625-1268  
smith376@umn.edu

Mohammed Javad  
Soleimani Pari  
Bu Ali Sina University  
Hamadan, Iran 65174  
98-811-823-5395  
98-811-422-7012  
j_soleimaniuk@yahoo.co.uk

Daryl Somers  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-984-4503  
204-983-4604  
somersd@agr.gc.ca

Ellen Sparry  
C & M Seeds  
Palmerston ON N0G 2P0  
519-343-2126  
519-343-3792  
esparry@redwheat.com

Jo-Ann Stebbing  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-983-0827  
204-983-4604  
jstebbing@agr.gc.ca

Terry Stepanik  
Acsion Industries Inc  
Pinawa MB R0E 1L0  
204-753-2255 x 2368  
204-753-8466  
stepanik@acsiion.com

Jeff Stewart  
Lethbridge Research Centre  
Lethbridge AB T1J 4B1  
403-317-2208  
403-317-2197  
stewartj@agr.gc.ca

Marcos Stulzer  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-984-0795  
204-983-4604  
mstulzer@agr.gc.ca

Gopal Subramaniam  
AAFC  
Ottawa ON K1A 0C6  
613-759-7619  
613-759-1701  
subramaniamra@agr.gc.ca

Ljiljana (Lily) Tamburic-Ilincic  
University of Guelph  
Ridgetown ON N0P 2C0  
519-674-1557  
519-674-1600  
litambur@ridgetownc.uoguelph.ca

Andy Tekauz  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-983-0944  
204-983-4604  
atekauz@agr.gc.ca

Julian Thomas  
Cereal Research Centre  
Winnipeg MB R3T 2M9  
204-984-3505  
204-983-4604  
jthomas@agr.gc.ca
Liyi Zhang  
Laval University  
Quebec QC  G1K 7P4  
418-656-2131 x 6397  
lyzhang68@yahoo.com

Yanfen Zheng  
Cereal Research Centre  
Winnipeg MB  R3T 2M9  
204-983-0936  
204-983-4604  
yzheng@agr.gc.ca