

Proceedings / Compte-rendu

4th Canadian Workshop on *Fusarium* Head Blight 4^e Colloque Canadien sur la *Fusariose*

**Ottawa Congress Centre
Ottawa, Ontario, Canada, November 1-3, 2005**

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**4th Canadian Workshop on *Fusarium* Head Blight
4^{ième} Colloque Canadien sur la *Fusariose*
Ottawa, Ontario, Canada, November 1-3, 2005**

Dear Workshop Participant

Welcome to the 4th Canadian Workshop on *Fusarium* Head Blight / 4^{ième} Colloque Canadien sur la *Fusariose* (CWFHB/CCF).

Since biennial meetings were initiated in 1999, the main priority of the CWFHB/CCF has been to provide a forum for information exchange among researchers, producers and industry. The 4th Workshop continues this tradition with overviews presenting advances in Breeding, Biotechnology, Epidemiology, Toxicology, Disease Management and Agronomy. The summaries of reviews and the abstracts in the following pages represent both our current understanding of *Fusarium* Head Blight (FHB) and the latest research findings on a disease that has cost the Canadian grain industry millions of dollars.

Many persons, institutions and companies have worked together to bring this meeting to fruition. We would like to acknowledge the financial contributions by *Fusarium* Action Canada and ACAA, and the coordinating efforts by Paul Thiel of Bayer Crop Science Inc. and Dr. Art Schaafsma of Ridgetown College, University of Guelph. This Workshop would not have been possible without those financial contributions. Thanks also are extended to all who have agreed to chair sessions and moderate discussions.

We wish to recognize the members of the local and national organizing committees whose willing and prompt responses to all the demands placed on them have made this Workshop possible. It has been a pleasure working with such a diligent and supportive team.

We trust you will take away memories of a meeting that brought together researchers, producers and industry members in a spirit of mutual learning and information exchange, to further our understanding of the many facets of FHB, and to identify the direction of future research.

Sincerely,

George Fedak and Harvey Voldeng
Co-chairs, 4th CWFHB/CCF

Program

Monday, October 31, 2005, HOTEL NOVOTEL (Next to Congress Centre)

6:00 - Registration Desk Open. (Entrance to Albion Room A-B, NOVOTEL)
10:00 pm Sandwich Buffet and Cash-Bar

Tuesday, November 1, 2005, OTTAWA CONGRESS CENTRE

7:15 am - Registration Desk Open (Capital Hall 1B-2B, Ottawa Congress
5:30 pm Centre)

8:15 am Opening Remarks
Yvon Martel, Acting Assistant Deputy Minister (Research), Agriculture and Agri-Food Canada

8:30 am Plenary Session (Capital Hall 1B-2B, Ottawa Congress Centre)
The Economic Impact of Mycotoxin
Felicia Wu, University of Pittsburgh and the RAND Corporation

SESSION I - BREEDING (Capital Hall 1B-2B, Ottawa Congress Centre)
Co-chairs: George Fedak and Harvey Voldeng

9:00 am Spring Wheat Overview
Stephen Fox, Agriculture and Agri-Food Canada

9:25 am Winter Wheat *Fusarium* Research in Canada - Progress and Up-date
Radhey Pandeya and Robert Graf, Agriculture and Agri-Food Canada

9:50 am Progress in Improving FHB Resistance in Barley
Bill Legge, Agriculture and Agri-Food Canada

10:15 - Health Break
10:45 am

10:45 am Improving FHB (Gibberella Ear Rot) Resistance in Maize
Lana Reid, Agriculture and Agri-Food Canada

11:10 am Fusarium Head Blight of Oat: Occurrence, Cultivar Responses and Research Update
Andy Tekauz, Agriculture and Agri-Food Canada

11:35 am General Discussion

12:00 - Lunch Buffet (Capital Hall Salon H, Ottawa Congress Centre)
1:30 pm

SESSION II - BIOTECHNOLOGY (Capital Hall 1B-2B, Ottawa Congress Centre)
Co-chairs: Linda Harris and Daryl Somers

1:30 pm Research Strategies towards Improving Wheat Quality by Resistance to *Fusarium* Head Blight
Viktor Korzun, EUREKA CEREQUAL PROJECT, Lochow-Petkus, Germany

2:00 pm Mapping FHB Resistance in Barley
François Belzile, Université Laval

2:30 pm Impact of Trichothecenes on Disease
François Eudes, Agriculture and Agri-Food Canada

3:00 - Health Break (Note: All authors to put up their posters in
3:30 pm Capital Hall 3B-4B)

3:30 pm Exploring the life cycle of *F. graminearum* using genomics
Frances Trail, Michigan State University

4:00 pm Genomics of Host Gene Expression
Gary Muehlbauer, University of Minnesota

4:30 pm General Discussion

5:00 - POSTER SESSION 1 (Capital Hall 3B-4B, Ottawa Congress Centre)
7.00 pm (Note: Authors of odd numbered posters should be present at their posters for this Poster Session.)
Snack Food and Cash Bar

Open Evening

Wednesday, November 2, 2005

7:15 am - 5:30 pm **Registration Desk Open (Capital Hall 1B-2B, Ottawa Congress Centre)**

SESSION III - EPIDEMIOLOGY (Capital Hall 1B-2B, Ottawa Congress Centre)

Co-chairs: Andy Tekauz and Jeannie Gilbert

8:30 am **Aerobiology and Regional Epidemiology of *Gibberella zeae***
Gary Bergstrom, Cornell University

9:00 am ***Fusarium* Head Blight: The Alberta Experience, 1995-2004**
Kelly Turkington, Agriculture and Agri-Food Canada

9:30 am **Previous Crop Residues and *Fusarium* Head Blight on Cereals**
Sylvie Rioux, CÉROM

10:00 - 10:30 am **Health Break**

10:30 am **A Trichothecene Chemotype Cline in Canada and the Changing Nature of FHB in North America**
Todd Ward, United States Department of Agriculture

10:55 am **Update on Studies for Biological Control of *Fusarium graminearum* / *Gibberella zeae* in Canada**
Jeannie Gilbert, Agriculture and Agri-Food Canada

11:15 am **CIMMYT FHB Research and International Collaborations**
Tomohiro Ban, Japan International Research Centre for Agricultural Sciences

11:30 am **General Discussion**

12:00 - 1:30 pm **Lunch Buffet (Colonel By Salon, Ottawa Congress Centre)**

SESSION IV - TOXICOLOGY (Capital Hall 1B-2B, Ottawa Congress Centre)

Co-chairs: Marc Savard and Art Schaafsma

1:30 pm **Toxicity, Exposure and Management of Risks from *Fusarium* Mycotoxins**
Tine Kuiper-Goodman, Health Canada

2:00 pm **International Legislation for Deoxynivalenol**
David Miller, University of Carleton

- 2:30 pm** **Recent Advances in the Toxicology of *Fusarium* Toxins**
Genevieve Bondy, Health Canada
- 3:00 -** **Health Break**
3:30 pm
- 3:30 pm** **Impact of Changing DON Guidelines on Grain Trade**
Tom Nowicki, Canadian Grain Commission
- 4:00 pm** **Update on the Mycotoxin Network**
Marc Savard, Agriculture and Agri-Food Canada
- 4:30 pm** **General Discussion**
- 5:00 -** **POSTER SESSION 2 (Capital Hall 3B-4B, Ottawa Congress Centre)**
7:00 pm *(Note: Authors of even numbered posters should be present at their posters for this Poster session.)*
Cash Bar
- 7:00 -** **Banquet (Colonel By Salon, Ottawa Congress Centre)**
10:00 pm
-

Thursday, November 3, 2005

SESSION V - DISEASE MANAGEMENT AND AGRONOMY

(Capital Hall 1B-2B, Ottawa Congress Centre)

Co-chairs: Art Schaafsma and Paul Thiel

- 8:30 am** **A Miller's Perspective**
Gordon Harris, Miller's Association
- 8:55 am** **A Western Grower's Perspective**
Gary Pike, Pike Management Group
- 9:20 am** **An Eastern Grower's Perspective**
Peter Gredig, Editor, Country Guide Magazine
- 9:45 am** **A Maltster's Perspective**
Phil de Kemp, Malting Industry Association of Canada
- 10:10 -** **Health Break**
10:40 am

Programme

Lundi, 31 Octobre, 2005, Hôtel NOVOTEL (à côté du Centre des congrès)

1800 - Inscription (à l'entrée de la salle Albion A-B du NOVOTEL)
2200h Buffet et Bar payant

Mardi, 1er Novembre, 2005, Centre des Congrès d'Ottawa

0715 - Inscription (Salle 1B-2B de la Capitale, Centre des congrès d'Ottawa)
1700h

0815h Remarques d'ouverture
Yvon Martel, sous-ministre adjoint intérimaire (Recherche), Agriculture et Agroalimentaire Canada

0830h Session Plénière
L'impact économique des mycotoxines
Felicia Wu, Université de Pittsburg et le Corporation RAND

SESSION I - AMÉLIORATION GÉNÉTIQUE (Salle 1B-2B de la Capitale)
Co-présidents-modérateurs : George Fedak et Harvey Voldeng

0900h Revue du blé de printemps
Stephen Fox, Agriculture et Agroalimentaire Canada

0925h Revue sur l'amélioration du blé d'automne
Radhey Pandeya et Robert Graf, Agriculture et Agroalimentaire Canada

0950h Progrès sur l'amélioration de la résistance de l'orge à la fusariose
Bill Legge, Agriculture et Agroalimentaire Canada

1015 - Pause-santé
1045h

1045h Progrès sur la résistance du maïs à la fusariose (gibérellose de l'épi)
Lana Reid, Agriculture et Agroalimentaire Canada

1110h Progrès sur la fusariose de l'avoine : Présence, réaction des cultivars et recherche
Andy Tekauz, Agriculture et Agroalimentaire Canada

1135h Discussion sur la session

1200 - Lunch buffet (Salon H de la Capitale, Centre des congrès)
1330h

SESSION II - BIOTECHNOLOGIE

Co-présidents-modérateurs : Linda Harris et Daryl Somers

1330h **Stratégies de recherche pour améliorer la qualité du blé par la résistance à la fusariose de l'épi**
Viktor Korzun, Projet EUREKA CEREQUAL, Lochow-Petkus, Allemagne

1400h **La carte génétique de la résistance de l'orge à la fusariose**
François Belzile, Université Laval

1430h **Impact des trichothécènes sur la maladie**
François Eudes, Agriculture et Agroalimentaire Canada

1500-1530h **Pause-santé (A noter : Tous les auteurs installent leurs affiches à la salle 3B-4B de la Capitale)**

1530h **Exploration du cycle de vie de *F. graminearum* en utilisant la génomique**
Frances Trail, Université de Michigan State

1600h **La génomique de l'expression génétique de la plante-hôte**
Gary Muehlbauer, Université de Minnesota

1630h **Discussions sur la session**

1700-1900h **SESSION I des AFFICHES (Salle de la Capitale 3B-4B)**
(A noter : Les auteurs d'affiches avec numéros impairs devraient être présents à leurs affiches)
Menu d'appoint et Bar payant

Soirée libre

Mercredi, le 2 Novembre 2005

**0715 - Bureau d'inscription à la Salle 1B-2B de la Capitale, Centre des congrès
1730h**

**SESSION III B ÉPIDÉMIOLOGIE (Salle 1B-2B de la Capitale, Centre des congrès)
Co-présidents-modérateurs : Andy Tekauz et Jeannie Gilbert**

0830h **Aérobiologie et épidémiologie régionale de *Gibberella zeae***
Gary Bergstrom, Université de Cornell

0900h **La fusariose de l'épi : L'expérience albertaine, 1995-2004**
Kelly Turkington, Agriculture et Agroalimentaire Canada

0930h **Les résidus des cultures précédentes et la fusariose de l'épi des
céréales**
Sylvie Rioux, CEROM

**1000 -
1030h** **Pause-santé**

1030h **Variation d'un cline chimiotypique de trichothécène au Canada et
évolution de la fusariose en Amérique du Nord**
Todd Ward, Ministère de l'Agriculture des Etats-Unis

1055h **Progrès sur le biocontrôle de *Gibberella zeae* / *Fusarium*
*graminearum***
Jeannie Gilbert, Agriculture et Agroalimentaire Canada

1115h **Recherche sur la fusariose et collaboration internationale au
CIMMYT**
*Tomohiro Ban, Japan International Research Centre for Agricultural
Sciences*

1130h **Discussions sur la session**

**1200 -
1330h** **Lunch buffet (au Salon Colonel By, Centre des congrès)**

**SESSION IV - TOXICOLOGIE
Co-présidents-modérateurs : Marc Savard et Art Schaafsma**

1330h **Toxicité, exposition et risques inhérents à la gestion des mycotoxines
fusariennes**
Tine Kuiper-Goodman, Santé Canada

1400h **La législation internationale sur le déoxynivalénol**

David Miller, University de Carleton

- 1430h** **Progrès récents sur la toxicologie des toxines fusariennes**
Geneviève Bondy, Santé Canada
- 1500 -** **Pause-santé**
1530h
- 1530** **Impact des changements de la réglementation du DON sur le marché des grains**
Tom Nowicki, Commission Canadienne des Grains
- 1600h** **Bilan du réseau satellite sur les mycotoxines**
Marc Savard, Agriculture et agroalimentaire Canada
- 1630h** *Discussions sur la session*
- 1700-** **SESSION II des AFFICHES (Salle de la Capitale 3B-4B)**
1900h *(A noter : Les auteurs d'affiches avec numéros pairs devraient être présents à leurs affiches).*
Bar payant
- 1900-** **Banquet (Salon du Colonel By, Centre des congrès)**
2200h
-

Jeudi, le 3 Novembre, 2005

SESSION V - MÉTHODES DE CONTRÔLE DE LA MALADIE ET AGRONOMIE
(Salle 1B-2B de la Capitale, Centre des congrès)
Co-présidents-modérateurs : Art Schaafsma et Paul Thiel

- 0830h** **Perspective des meuniers**
Gordon Harris, Association des Meuniers
- 0855h** **Perspective d'un producteur de l'ouest canadien**
Gary Pike, Pike Management Group
- 0920h** **Perspective d'un producteur de l'est canadien**
Peter Gredig, Editeur du Magazine Country Guide
- 0945h** **Perspective des malteurs**
Phil de Kemp, Association des Malteurs
- 1010 -** **Pause santé**
1040h

1040h **Perspective de l'industrie de l'alimentation animale**
Martin Clunnie, Grand Valley Fortifiers

1105h **Perspective des opérateurs de silos à grains**
Richard Wansbutter, Saskatchewan Wheat Pool

1130 - **Discussions de synthèse**
1230h

1230h **Remarques finales des co-présidents du colloque**

1330*- **Réunion du réseau satellite sur les mycotoxines**
1500h *Marc Savard, Agriculture et agroalimentaire Canada*
* **Heure prévue**

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§ Saskatchewan Wheat Pool

§ Syngenta Crop Protection

Speaker Profiles

Felicia Wu, Assistant Professor, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh.

Felicia Wu's research interests include economic and policy analysis applied to food safety, biotechnology, mycotoxins, and indoor environmental quality. She is chair-elect of the Food/Water Specialty Group and the Risk Communication Specialty Group of the Society for Risk Analysis. Recently she co-chaired an international conference 'Developing Policies to Improve Indoor Environmental Quality: Transatlantic Viewpoints.' She has served as a proposal and manuscript reviewer for the US Department of Agriculture, the National Science Foundation and several journals. She is the author of a number of publications concerning genetically modified food, mycotoxins, environmental risk assessment and regulation, and public education and communication. Felicia Wu holds a Ph.D. in Engineering and Public Policy from Carnegie Mellon University and an A.B. in Applied Mathematics / Medical Sciences from Harvard.

Stephen Fox, Wheat Breeder, Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba.

Stephen Fox has been a wheat breeder at AAFC's Cereal Research Centre in Winnipeg since 2001. His work is focused on the development of new and improved varieties of Canada Western Red Spring (CWRS) wheat. He is primarily concerned with developing high-yielding lines with excellent disease resistance, pre-harvest sprouting resistance and end-use quality, primarily for baking markets. Stephen has authored or co-authored several research papers and abstracts on wheat, barley and oat genetics, molecular genetics and plant pathology. From the University of Manitoba, he holds a M.Sc. in oat genetics and a Ph.D. in barley genetics and pathology. Stephen was a postdoctoral fellow studying oat molecular genetics at the University of Minnesota in St. Paul, MN and a held another postdoctoral position with AAFC in Swift Current, SK. studying preharvest sprouting resistance in wheat. Prior to joining AAFC, Stephen was a durum wheat breeder for four years at the University of Saskatchewan in Saskatoon.

Bill Legge, Research Scientist, Brandon Research Centre, Agriculture and Agri-Food Canada, Brandon, Manitoba.

Bill Legge obtained his Ph.D. from the University of Manitoba in 1987, and has been working at the Brandon Research Centre since that time. His main area of research has been two-row malting barley breeding and he has released a number of cultivars including AC Metcalfe and Newdale. Bill has been leading a project to improve FHB resistance in barley since 2000.

Lana Reid, Corn Breeder, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario.

Lana Reid leads a team of researchers developing new corn inbreds and populations with early maturity, cold tolerance and pest resistance. Her team has released the only publicly available corn inbreds with improved resistance to gibberella ear rot (*Fusarium graminearum*). Lana Reid has been with Agriculture and Agri-Food Canada since

graduating from McGill University with a Ph.D. in plant genetics and pathology in 1991. She received her M.Sc. and B.Sc. from the University of Ottawa. She has published more than 60 research papers, the majority of which focused on her research with *Fusarium* fungi.

Andrej (Andy) Tekauz, Plant Pathologist, Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba.

Andy Tekauz, studies *Fusarium* Head Blight (FHB) and leaf spot diseases of barley and oat re. their management by resistance and other means. He is the Head of Cereal Diseases at the Cereal Research Centre and did the initial studies on FHB in wheat, barley, and most recently, in oat, during the current and ongoing FHB epidemic in western Canada dating back to 1986. He will describe the status of FHB in oat in western Canada, cultivar responses to the disease, and the prospects of breeding for resistance in his presentation.

François Eudes, Cereal biotechnologist, Bioproducts & Bioprocesses, Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta.

François Eudes completed his Ph.D. on *Fusarium* Head Blight in wheat in 1998, at l'Université Laval, QC. He joined Agriculture and Agri-Food Canada, Lethbridge soon thereafter. In 2001, François resumed his research activities in wheat FHB with a provincial grant. In collaboration with breeders he has applied the *in vitro* selection process and disease screening to four prairie wheat classes and diverse sources of resistance, and initiated germplasm release. This applied activity is ongoing. The research focus at the Lethbridge Centre has shifted from the role of trichothecenes in plant pathogen interaction to mechanisms of wheat and barley resistance/tolerance to these toxins.

Frances Trail, Associate Professor, Plant Biology and Plant Pathology, Michigan State University, East Lansing, Michigan.

Frances Trail has been investigating *Fusarium* Head Blight in wheat for the past nine years. Her research focuses on development, particularly sexual development in *Fusarium graminearum*. Two focal points in her research have been the elucidation of the mechanism of forcible ascospore discharge and characterization of the function of polyketide synthase genes in the head blight fungus. She has recently been using the Affymetrix genome chip for *F. graminearum* to understand shifts in gene expression during development and differentiation both in culture and in the host.

Gary Muehlbauer, Associate Professor, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota.

Gary Muehlbauer obtained his Ph.D. from the University of Minnesota and worked as a postdoctoral research associate at the University of California at Berkeley. One primary area of focus in his lab is studying *Fusarium* head blight of the small grains barley and wheat. He and his colleagues are actively engaged in exploring these plant-pathogen interactions. They are using genetics, molecular biology, genomics and plant transformation technologies to understand these interactions and to develop FHB resistant barley and wheat.

Sylvie Rioux, Plant Pathologist, Centre de recherche sur les grains (CÉROM) Sainte-Foy, Quebec.

Sylvie Rioux has been working as plant pathologist at CÉROM since 1998. CÉROM is a research center on grains provided by MAPAQ (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec), FPCCQ (Fédération des producteurs de cultures commerciales du Québec), and La Co-op fédérée. She works mostly on FHB in cereals and on sclerotinia stem rot in soybean. She is in charge of the Saint-Hyacinthe FHB nursery and has collaborated on many projects, such as some conducted at the Normandin Farm (Agriculture and Agri-Food Canada). Sylvie, in collaboration with Denis Pageau, Julie Lajeunesse, and Jean Lafond from AAFC-Normandin, will present studies conducted to measure the effect of crop rotation and tillage practices on FHB of cereals.

Todd Ward, Microbial Genomics Research Unit, USDA/ARS/NCAUR, Peoria, IL.

Todd Ward's work with FHB has primarily focused on the evolution of trichothecene mycotoxins and the trichothecene biosynthetic gene cluster. This work has led to the discovery that genetic polymorphism within the trichothecene gene cluster of FHB pathogens has been maintained through multiple speciation events by balancing selection for chemotype differences. Additional work on FHB has focused on understanding species limits, the molecular evolution of the mating type locus, and molecular epidemiology of FHB pathogens and trichothecene toxins.

Jeannie Gilbert, Plant Pathologist, Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba.

Jeannie Gilbert has been working on FHB on wheat for 16 years and has collaborated on more than 30 funded projects since 1996. She was project leader on 15. The work has improved the understanding of the effect of weather on FHB of wheat, effect of the disease on yield and quality, duration of pathogen survival on wheat kernels, refined disease screening protocols etc. She investigates control and management strategies for *Fusarium* head blight (FHB) of wheat. She will present information on the current knowledge of control and management strategies for FHB excluding the areas of chemical control and breeding and will include rotations, tillage practice, biocontrol, under-storey cover crops, irrigation and stubble management.

J. David Miller, NSERC Industrial Research Chair on Fungal Toxins and Allergens, Institute of Biochemistry, Carleton University, Ottawa, Ontario.

J. David Miller joined Agriculture Canada in 1982 and was head of the *Fusarium* mycotoxin program at the Plant Research Centre from 1988-1997. In 1997, he joined the Institute of Biochemistry at Carleton University where he is NSERC Industrial Research Chair on fungal toxins and allergens. He has published >250 papers on fungi and fungal toxins, co-edited six books, served on IARC Monographs 56 (1992) and 86 (2002) which included consideration of 11 mycotoxins well as an IARC STP on mycotoxins (2003-5), was on the drafting committee of the WHO IPCS monograph on fumonisin B1. From 1995-1999, he was a member of the FDA Committee that approves studies nominated by the FDA to the National Toxicology Program and is a member of the Toxicology Forum (Washington), serving on its Board of Directors (1993-). He has received the Agriculture

and Agri-Food Canada AgEcellence Award (1992), the Toxicology Forum Scott Award (1998), and the Ottawa Life Sciences Council Applied Research Award (2002).

Genevieve Bondy, Head, Genotoxicity and Carcinogenicity Section, Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario.

Genevieve Bondy received a Ph.D. in toxicology from the University of Guelph, studying the haematological effects of trichothecene mycotoxins. Following her graduate training she conducted postdoctoral research on deoxynivalenol immunotoxicology at Michigan State University. Her current research projects at Health Canada focus on the immunomodulatory effects of foodborne environmental contaminants and genetically modified or novel foods, and on assessing the carcinogenicity and immunotoxicity of fungal toxins using transgenic rodent models as an alternative to the traditional rodent 2-year cancer bioassay.

Marc Savard, Research Scientist, Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Central Experimental Farm, Ottawa, Ontario.

Marc Savard is working on mycotoxins as a member of the Food Safety and Quality group. He has been involved in many aspects of mycotoxin research for twenty years, including the biosynthesis, the synthesis, the structure determination, the development of analytical methods, the analysis, the chemistry and the decontamination of mycotoxins. Marc was also involved in the birth of the mycotoxin network, a network of researchers from AAFC, Health Canada, CFIA and elsewhere, involved in the study of mycotoxins.

Marc Savard est un chercheur à Agriculture et Agroalimentaire Canada, où il travaille sur les mycotoxines en tant que membre de du groupe Salubrité et Qualité des aliments. Il a été impliqué dans plusieurs aspects de la recherche sur les mycotoxines depuis vingt ans, incluant la biosynthèse, la synthèse, la caractérisation, le développement de méthodes analytiques, l'analyse, la chimie et la décontamination des mycotoxines. Marc a aussi été impliqué dans la naissance du réseau des mycotoxines, un réseau de chercheurs d'AAFC, Santé Canada, l'AIAC et d'ailleurs impliqués dans l'étude des mycotoxines.

SESSION I - BREEDING

Winter Wheat *Fusarium* Research in Canada - Progress and Up-date

Radhey Pandeya, Agriculture and Agri-Food Canada

Progress in Improving FHB Resistance in Barley

Progrès dans l'amélioration de la résistance à la *fusariose* chez l'orge

Bill Legge, Agriculture and Agri-Food Canada

Improving FHB (Gibberella Ear Rot) Resistance in Maize

Lana Reid, Agriculture and Agri-Food Canada

***Fusarium* Head Blight of Oat: Occurrence, Cultivar Responses and Research Update**

Andy Tekauz, Agriculture and Agri-Food Canada

Winter Wheat *Fusarium* Research in Canada - Progress and Up-date

Radhey Pandeya

Agriculture and Agri-Food Canada, Ottawa, ON.

Pandeya, R.¹; Graf, R.²; Matthew, P.¹; Kalikililo, A.¹

¹ Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON.; ² Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB.

Fusarium Head Blight (FHB) has become a national problem. It continues to cause economic and product quality/safety damage to the Canadian wheat crop. Continued development of FHB resistant winter wheat is of vital importance in Ontario, Quebec and Atlantic Canada. Despite the likelihood of escape in western Canada, incorporation of FHB resistance into winter wheat is important, particularly for the moist eastern prairies. Furthermore, the incorporation of FHB resistance into new CWRW cultivars may allow national registration for all parts of Canada. Food/feed safety and sustainability of the overall wheat agri-economy in Canada (and other major wheat producing regions in USA, Asia, Europe and Australia) have been affected and/or impaired by mycotoxin residues on grains and grain products due to a frequently occurring fungal disease, *Fusarium* Head Blight (FHB) caused by *Fusarium graminearum*.

This FHB, a fungal disease, disrupts seed development, lowers yield and renders harvested grains unfit for human and animal consumption. The devastating effect of FHB persists through every part of the wheat value chain, including seed, production, trade, feed, food, processing, domestic and international trade, and ultimately the health of consumers. The cumulative loss since the 1990's to the direct farm-gate value of Canadian wheat exceeds well over \$1.5 billion. The USA has reported losses over \$3.2 billion. A recent FHB epidemic in 2004 devastated winter wheat in Ontario. FHB has been spreading throughout the Canadian provinces since the 1980's. Health Canada and the international organization, CODEX, are establishing new lower limits for mycotoxin residue on grain and grain products, which would be extremely difficult to comply with in years of higher FHB infection. Therefore, it becomes imperative to develop and deploy resistant wheat cultivars in order to safeguard our wheat grain at the production stage by providing FHB resistant cultivars to producers and the grain industry, and help maintain and enhance the sustainability of the Canadian wheat agri-economy of \$4.5 billion/yr.

We have made progress at Agriculture and Agri-Food Canada- Eastern Cereal and Oilseed Research Centre, in cooperation with Hyland Seeds of W.G. Thompson Limited. We have developed and released the first Soft Red Winter Wheat in (2001-02), FT Wonder- the first wheat resistant/tolerant to FHB in North America that has the lowest DON levels and visual symptoms. The commercial seed-sale and production has already begun. In 2005, Agriculture and Agri-Food Canada- Eastern Cereal and Oilseed Research Centre has registered the two first FHB resistant/tolerant soft white winter wheat cultivars, FT-Action and Ashley in Canada (and anywhere in the world) in

cooperation with Hyland Seed (under a MII agreement). Breeders' Seed production and multiplication are underway. Agriculture and Agri-Food Canada- Eastern Cereal and Oilseed Research Centre is working with SeCan to develop FHB wheat cultivars as well. The detailed presentation will highlight the R&D findings and activities at Federal, Provincial and private sector organizations in Canada.

Progress in Improving FHB Resistance in Barley

Bill Legge

Brandon Research Centre, Agriculture and Agri-Food Canada, Brandon, MB.

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Encouraging progress has been made in improving resistance to fusarium head blight (FHB), the most economically destructive disease of barley in Canada. In eastern Canada, several two-row feed cultivars with improved FHB resistance, including Island and more recently AB241-1, have been released. These cultivars were developed from existing variation within the Agriculture and Agri-Food Canada (AAFC) Ottawa breeding program. In western Canada, advanced barley lines with improved FHB resistance over AC Metcalfe began to enter the Western Cooperative Two-row Barley Registration Test in 2004, with the two-row feed TR04378 developed from a standard breeding cross at the Crop Development Centre, Saskatoon, being particularly noteworthy. Also entering this test in 2004 and 2005 were five two-row malting lines developed at AAFC Brandon from crosses involving 'exotic' FHB resistance sources Harbin and Svanhals, along with two lines produced through *in vitro* selection. Two-row hullless cultivars have shown among the lowest deoxynivalenol (DON) levels, and further progress appears possible. Progress has lagged in six-row barley with hullless lines showing the most promise. Since FHB resistance is a quantitatively inherited trait strongly influenced by environment, testing over numerous locations and years and access to adequate DON testing capacity will be required for continued progress.

Keywords: *Fusarium graminearum*, *Fusarium* head blight, barley, *Hordeum vulgare*, genetic resistance, cultivars

Progrès dans l'amélioration de la résistance à la fusariose chez l'orge

Des progrès encourageants ont été obtenus sur l'amélioration de la résistance à la fusariose des panicules (FP), la maladie de l'orge la plus économiquement destructive au Canada. Dans l'est du Canada, plusieurs cultivars d'alimentation à deux rangs avec une résistance améliorée à la FP, y compris Island et plus récemment AB241-1, sont maintenant disponibles. Ces cultivars ont été développés à partir de la variation existant au programme d'Agriculture et Agroalimentaire Canada (AAC) d'Ottawa. Dans l'ouest

du Canada, les lignées avancées d'orge avec la résistance à la FP meilleure que celle d' AC Metcalfe ont commencé à être incorporées dans l'essai coopératif de l'ouest pour l'enregistrement de l'orge à deux rangs en 2004, avec l'orge d'alimentation TR04378 à deux rangs, développé d'un croisement standard au centre de développement des cultures de Saskatoon, étant particulièrement remarquable. D'autres participants à cet essai en 2004 et 2005 étaient cinq lignées de maltage à deux rangs développées à AAC Brandon par des croisements impliquant les sources de résistance à la FP "exotiques" Harbin et Svanhals, avec deux lignées produites par le choix in vitro. Les cultivars d'orge nus à deux rangs ont montré parmi les niveaux les plus bas de désoxynivalénol (DON), et de meilleurs résultats semblent possibles. Le progrès n'est pas aussi bon avec l'orge à six rangs où les lignées nues montrent le plus de promesse. Puisque la résistance à la FP est un trait quantitativement hérité fortement influencé par l'environnement, les lignées doivent être testées à plusieurs locations pendant plusieurs années et l'accès à une capacité constante de dosage pour le DON seront nécessaires pour le progrès continu.

Mots clés : *Fusarium graminearum*, Fusariose des panicules, orge, *Hordeum vulgare*, résistance génétique, cultivars

Improving FHB (Gibberella Ear Rot) Resistance in Maize

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Three mycotoxin-producing species of *Fusarium* are predominantly responsible for ear rot of maize or corn in Canada: *F. graminearum*, *F. verticillioides* and *F. subglutinans*. Since 1986, an ear rot resistance breeding program has been conducted by Agriculture and Agri-Food Canada in Ottawa. Most of the breeding work has focussed on *F. graminearum*. Eight inbred lines with improved resistance have been released. These inbreds differ in their genetic backgrounds, levels and types of resistance (as related to mode of fungal entry, ie. silk vs kernel), mechanisms of resistance, and combining ability. The current breeding strategy is to combine these inbreds and to improve the agronomy of the highly resistant inbreds, as we have now exhausted all known adapted sources of resistance. We are also investigating an apparent indirect selection for resistance to *F. verticillioides* and common smut, *Ustilago zaeae*. Collaborative work with the University of Guelph (P. Pauls) and the University of Ottawa (J.T. Arnason) has led to the identification of genetic markers for both silk and kernel resistance. A maize gene that codes for a putative 267-amino acid guanylyl cyclase-like protein (ZmGC) has been characterized and associated with *F. graminearum* resistance (P. Pauls and Y. Jiazheng).

***Fusarium* Head Blight of Oat: Occurrence, Cultivar Responses and Research Update.**

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Fusarium head blight (FHB) has been recognized as a widespread disease of oat in Manitoba only since 2002, following the first systematic, contemporary survey for FHB in the crop. The presence of FHB in 36 to 100% of the oat fields surveyed from 2002 to 2005, and the isolation of *Fusarium graminearum* and other FHB-causing *Fusarium* spp. from sampled kernels, indicated that FHB has become an important disease of the crop. Varietal selection is a strategic component of an integrated strategy for the management of FHB in other cereals such as wheat and barley. Therefore, registered oat cultivars, both hulled and hullless, were tested in field trials from 2001 to 2003 to obtain information on their reactions to FHB, and to identify any differential responses to the disease. In each year, trials were seeded at three locations in southern Manitoba, and consisted of plots of 15, 17 or 20 oat cultivars, replicated three times. FHB reactions were determined by assessing levels of *fusarium* damaged kernels (FDK), *Fusarium* spp. kernel infestation, and of the mycotoxin, deoxynivalenol (DON). Testing on a smaller scale was continued in 2004 and 2005 to obtain additional site-year data for newly-registered cultivars. Based on 2001-2003 results, FDK levels averaged 6.7% (range 3.7-9.5%), total levels of *Fusarium* spp. (mainly *F. graminearum*, *F. poae*, *F. avenaceum* and *F. sporotrichioides*) averaged 18.8% (range 6.1-26.0%), *F. graminearum* levels averaged 5.3% (range 0.6- 9.1%) and DON levels averaged 2.2 ppm (range 0.8-3.7 ppm). Average and maximum levels all were generally lower than those for the wheat and barley check cultivars included in the field trials. Among the oat cultivars tested over at least 6 site-years (n = 17), DON levels were significantly ($P = < 0.05$) lower in 'Furlong' oat, compared to cvs. such as 'CDC Boyer', 'AC Assiniboia', 'Triple Crown', and others. However, no correlativity was found for other FHB components measured, although significant differences occurred among cultivars for levels of FDK, total *Fusarium* and *F. graminearum*. Based on data sets from 2003 and 2004 examined individually, the newly-registered hullless cv 'Boudrias' appeared to have the best over-all reaction (lowest levels for most components) to FHB. Other hullless cultivars such as 'AC Belmont', 'AC Gwen', and 'Lee Williams' also had numerically lower values for FHB components. The hullless character is known to correlate with substantial reductions in measured levels for both *Fusarium* spp. and DON, as > 50% of these occur in the hulls that are 'lost' during harvest. Additional confirmatory information on cultivar responses will be available after the data from the 2005 trials is analysed and combined with previous results.

The annual and site variation in reactions to FHB and the relatively small overall

differences in oat cultivar responses to the various components of FHB (taking the hulless factor into account), suggest there is little genetic variability for resistance to FHB in western Canadian oat germplasm. As such, alternative, superior resistance will need to be sought in exotic cultivated oat germplasm and/or that of wild relatives. These studies began in 2005, and several hundred accessions from several regions of the planet were planted in an FHB Disease Nursery at Portage la Prairie, Manitoba. Analyses for *Fusarium* and DON levels will be conducted to detect any promising source(s) for use in a crossing program to improve the FHB resistance in oats adapted for western Canada.

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SESSION I - BREEDING

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Vigier, B.J.

Poster 1.

Significant cultivar x location interactions for deoxynivalenol contamination in barley

Choo, T.M.¹; Vigier, B.¹; Etienne, M.²; Sparry, E.³; Caldwell, C.D.⁴; Falk, D.E.⁵; Savard, M.E.¹; Martin, R.A.⁶

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Fusarium head blight (FHB) is a devastating disease of barley (*Hordeum vulgare*) in Eastern Canada. It gives rise to accumulation of deoxynivalenol (DON) in the grains. FHB was epidemic in the Maritimes and Ontario in 2004. Therefore, seed samples from field trials were collected at Harrington (Prince Edward Island), Kentville (Nova Scotia), Elora (Ontario), Harriston (Ontario, Monkton (Ontario) to study the response of barley cultivars to DON accumulation at different locations. DON concentration was higher in Ontario than in the Maritimes. In the Maritimes, the average DON concentration was higher at Harrington than at Kentville (3.37 vs. 0.28 mg kg⁻¹ for six-row cultivars, 0.83 vs. 0.26 for two-row cultivars). The 15 two-row cultivars significantly differed in DON concentration (0.35 to 1.10 mg kg⁻¹) only at Harrington, while the 20 six-row cultivars did not differ in DON concentration at both locations. The average DON concentration was not different between Monkton (5.1 mg kg⁻¹) and Harriston (5.0 mg kg⁻¹), but the relative DON concentration of these 37 cultivars varied significantly between the two locations. Two-row cultivars, on average, contained less DON than six-row cultivars (2.5 vs. 6.8 mg kg⁻¹). The average DON concentration at Elora was 2.2 mg kg⁻¹. Correlation coefficients between replicates (four replicates at each location) were significant at Monkton ($r = 0.45$, $P < 0.01$ to $r = 0.87$, $P < 0.01$) and Harriston $r = 0.73$, $P < 0.01$ to $r = 0.79$, $P < 0.01$). These results indicate that cultivar x location interactions for DON contaminations were significant in barley where natural infection of FHB was high. Therefore, data from more than one location are needed to assess the resistance level of barley cultivars.

Poster 2.

Genetic diversity among *Fusarium* species in Quebec.

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Recently, we have determined the distribution and prevalence of the *Fusarium* complex species associated with *Fusarium* head blight in barley for the years 2000-2001-2002 in twelve administrative regions of the province of Québec. Three species were responsible for most of the infections: *F. graminearum* (41.0 %), *F. poae* (23.1 %) and *F. avenaceum* (15.8%). Isolates (~25) of each of the major species were further analyzed by sequencing a portion of the translation elongation factor 1 α (TEF1 α) gene and comparing these sequences to those found in the FUSARIUM ID database. To study the genetic diversity and structure within these *Fusarium* species, we collected data on the polymorphism at VNTR and ISSR markers. A phylogenetic analysis was conducted based on the genetic distances (using Nei and Li's coefficient). Subsequent analyses will be conducted to measure population differentiation, gene flow and linkage disequilibrium between each population. Knowledge of genetic structure within *Fusarium* species will be useful in defining the risk of pathogen evolution as well as benefiting disease management strategies in barley breeding programs.

Poster 3.

Anti - *Fusarium* activity assessment of cationic peptides using AlamarBlue™ assay

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The production of mycotoxins by *Fusarium* species causes *Fusarium* Head Blight (FHB) in cereals and renders grain unsuitable for human consumption. Certain antimicrobial cationic peptides have demonstrated antifungal activity against a broad spectrum of plant pathogens including *Fusarium* species. The potential exists to express cationic peptides in transgenic wheat plants, rendering them resistant to FHB and other diseases. This work compares the *in vitro* efficacy of six different cationic peptides on a variety of *Fusarium* strains that affect wheat crops in Canada. The AlamarBlue™ assay was used to test the viability of *Fusarium* spores from different species that were treated with 10-100 µg/ml of cationic peptides. The cationic peptides MsrA2 (the dermaseptin variant), MsrA3 (the temporin analog), 10R and 11R (the indolicidin variants), PV5 (the polyphemusin variant) and BMAP-18 (the truncated form of BMAP-27) were tested. Our results show that these peptides have varying cytotoxic effects on the *Fusarium* strains tested, but no single peptide was effective for killing all the tested strains. Thus, our results suggest that combinations of these peptides may show promise for use in transgenic wheat crops by providing a broad-spectrum resistance against *Fusarium* strains.

This research was supported by the NCE-AFMnet and AAFC grant to S. Misra.

Poster 4.

Assessment of *Fusarium* Head Blight artificial inoculation methods and deoxynivalenol levels in barley lines

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Fusarium Head Blight (FHB), caused by *Fusarium graminearum* (Schwabe) is one of the most devastating diseases affecting the production of barley causing drastic decreases in crop yield and grain quality. Deoxynivalenol (DON), produced by *F. graminearum*, is a factor of virulence in the plant pathogen interaction. Nineteen barley lines representing various FHB level of resistance were point inoculated or spray inoculated in the greenhouse at anthesis stage with 40,000M/ml *F. graminearum* macroconidia. Following inoculation, barley plants were kept in dedicated greenhouse at 21/24EC and 95% humidity for 3 days to favour disease establishment and were then returned to normal growing conditions and greenhouse at 18/21EC without humidity control (about 45%). Eighteen days post-inoculation heads were collected and spikelets displaying FHB symptoms were rated for progression of the disease. The 19 barley lines were also evaluated in field trials in a Brandon, MB nursery from 2000-2004. An analysis of disease progression and DON levels reported from field trials was done and compared to the ones done indoor. Statistical analysis indicated that barley lines representing candidate sources of FHB resistance or FHB susceptible sources showed quite consistent behaviour in all three artificial methods. Barley lines representing intermediate FHB resistance displayed varying degrees of symptoms in the three treatments. Artificial inoculation methods and DON quantification enable us to rate the level of resistance of the barley lines, with confidence. Reproducibility of methods of evaluation will be discussed.

Keywords: barley, *Fusarium* head blight, mycotoxin, deoxynivalenol, artificial inoculation method

Evaluation de méthodes d'inoculation artificielle pour la fusariose de l'épi et la teneur en désoxynivalénol chez des lignées d'orge

La fusariose de l'épi, causée par *Fusarium graminearum* (Schwabe) est l'une des maladies les plus dévastatrices pour la production d'orge, entraînant des pertes de rendement et de qualité importantes. La désoxynivalénol (DON), produite par *F. graminearum*, est un facteur de virulence de l'interaction plante pathogène. Dix neuf lignées d'orge représentant divers degrés de résistance ont été inoculé par la méthode d'injection et d'aspersion en serre, au stage d'anthèse, avec un inoculum à 40 000 macroconidia de *F. graminearum* par ml. Suite à l'inoculation, les plants ont été maintenu dans une serre aménagée à 21/24 EC et 95% d'humidité pendant trois jours afin de favoriser l'établissement de la maladie. Puis, les plantes ont été ramenées dans une autre serre dans les condition normale de culture à 18/21 EC sans contrôle du taux d'humidité (environ à 45%). Dix huit jours après l'inoculation, les épis ont été récoltés et les symptômes notés. Ces 19 lignées ont aussi été évaluées en champ à la nurserie de

Brandon, MB., de 2000 à 2004. Une analyse de la progression de la maladie et de la teneur en DON en situation de champ a été comparée à celles réalisées avec les données générées en serre. Les analyses statistiques indiquent que les lignées d'orge résistantes et sensibles présentent un comportement semblable sous les diverses méthodes d'évaluation. Les lignées de résistance intermédiaire présentaient des degrés de résistance variable selon la méthode utilisée. Les méthodes d'inoculation artificielle et la quantification de la teneur en DON nous permettent de mesurer avec confiance le degré de résistance de ces lignées d'orge. La reproductibilité des méthodes d'évaluation sera discutée.

Mots clés : orge, *Fusariose* de l'épi, mycotoxine, désoxynivalénol, méthode d'inoculation artificielle

Poster 5.

Proteomic analysis of *Fusarium graminearum* proteins detected in the *Fusarium* head blight infected spikes of hexaploid wheat (*Triticum aestivum*)

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To better understand fungal development and pathogenicity of *Fusarium graminearum* in the early wheat spikes infection, we compared protein profiles of FHB infected and control spikelets among six cultivars exhibiting various levels of FHB resistance using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) system. Twelve spots present only in FHB infected spikelets 72 h post inoculation were detected and identified by liquid chromatograph tandem mass spectrometry. Eight of the twelve spots were consistently present in all six cultivars and can be used as biomarkers for FHB infection. Based on reported identification of polypeptides using LC-MS/MS, three enzymes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), mannitol dehydrogenase, and superoxide dismutase, might be related to the pathogenicity in the process of infection. Two membrane proteins, an outer membrane protein II and a putative integral membrane protein, and a protein similar to member I of H3 histone family were also identified. The remaining six spots were identified as five different hypothetical proteins in NCBI. They were found to carry conserved domains of COG3812, an uncharacterized protein conserved in bacteria; pfam00254, an FKBP-type peptidyl-prolyl *cis-trans* isomerase; cd01132, an F1 ATP synthase; pfam01459, a porin 3; and pfam00082, a peptidase S8. Possible functions of these pathogenicity related proteins are also discussed.

Keywords: FHB, 2D-PAGE, LC-MS/MS

Détection de protéines de *Fusarium graminearum* dans les épis infectés par la fusariose chez le blé (*Triticum aestivum*)

Afin de mieux comprendre le développement et la pathogénicité de *Fusarium graminearum* lors de l'infection des épis chez le blé, nous avons comparé par électrophorèse à deux dimensions (2D-PAGE), les profils de protéines des épis contrôles et infectés de six variétés exhibant différents niveaux de résistance à la fusariose. Douze protéines présentes seulement dans les épis infectés 72 h après l'inoculation ont été détectées et identifiées suite à une analyse par chromatographie liquide couplée à un spectrophotomètre de masse. Huit des douze protéines étaient présentes chez tous les cultivars et peuvent être donc utilisées comme marqueur biologique de la fusariose. Trois enzymes, la glyceraldéhyde-3-phosphate déshydrogénase (GAPDH), la mannitol déshydrogénase et la superoxyde dismutase pourraient être impliquées dans la pathogénicité de la fusariose. Deux protéines membranaires et histone H3 ont été identifiées. L'identité des six autres protéines n'a pas été identifiée de façon définitive. Par contre, des domaines fonctionnels ont été identifiés suggérant des rôles possibles: une protéine conservée mais de rôle inconnu chez les bactéries; une FKBP peptidyl-prolyl *cis-trans* isomérase; une ATP synthase de type F1; une porine de type 3; et une peptidase de type S8. Les fonctions potentielles de ces protéines reliées à la présence de la fusariose seront discutées.

Mots clés : Fusariose de l'épi, 2D-PAGE, LC-MS/MS

Poster 6.

Introgression of *Fusarium* head blight (FHB) resistance in Canadian wheat germplasm by *in vitro* and marker-assisted selections

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Fusarium head blight (FHB), incited by *Fusarium graminearum*, causes important yield and quality losses in wheat in Canada and worldwide. The development of wheat cultivars with resistance to FHB is difficult to achieve due to the laborious screening methods and the complex mechanisms of resistance, both of which are subject to environmental variability. QTLs for FHB severity and DON level provide the basis for marker-assisted selection of resistant lines. We have developed *in vitro* selected doubled haploid (DH) lines from different crosses between Canadian cultivars AC Superb (CWRS), AC Crystal (CPS) and candidate sources of resistance from China, Brazil and CIMMYT. The response of the DH lines and their parental lines to FHB infection was also evaluated using point inoculation method in greenhouse and spray method in field conditions. Molecular analyses were conducted on the parental lines and the genetic material derived from, using simple sequence repeat (SSR) markers linked to FHB resistance QTLs identified from the literature. We will present data on FHB assessment of parental and doubled haploid progeny, and the use of four polymorphic QTLs in Canadian germplasm. Molecular analyses have allowed us to identify resistant DH lines with the same haplotype as the resistant parent. The application of these QTLs in Canadian wheat germplasm will be discussed. Based on these results, we believe that *in vitro* selection combined with marker-assisted selection, SSR markers, could offer a faster and more efficient way of developing FHB resistant wheat cultivars.

Keywords: wheat, *Fusarium* head blight, *in vitro* selection, mycotoxin, doubled haploids, deoxynivalenol, molecular marker

Introgression de la résistance à la fusariose de l'épi chez le germoplasme de blé canadien par sélection *in vitro* et à l'aide de marqueurs moléculaire

La fusariose de l'épi causée par *Fusarium graminearum* est à l'origine de pertes de rendement et de qualité importantes chez le blé, au Canada et dans le monde. La création de cultivars de blé avec résistance à la fusariose de l'épi est difficile à réaliser du fait de la complexité des mécanismes de résistance et des méthodes de sélection laborieuses, tous deux sujet aux variations du à l'environnement. Les QTLs et les marqueurs associés à la teneur en désoxynivalénol et la résistance à la maladie sont de bons outils pour accompagner la sélection. Nous avons produit des lignées de blé haploïdes doubles (HD), sélectionnées *in vitro*, à partir de divers croisements entre les cultivars canadien AC Superb (CWRS), AC Crystal (CPS) et des sources de résistance potentielle provenant de Chine, du Brésil ou du CIMMYT. La réponse des lignées HD et des parents à la fusariose de l'épi a été évalué par les méthodes d'injection, en serre et d'aspersion en champ. Des analyses moléculaires ont été réalisées sur ce même matériel à l'aide des marqueurs SSR associés aux principaux QTLs décrits dans la littérature scientifique. Nous présentons les résultats d'évaluation de la résistance chez les parents et les haploïdes

doublés, et l'utilisation de quatre QTLs polymorphiques. Ces analyses moléculaires ont permis d'identifier des lignées HD résistantes avec le même haplotype que le parent résistant. L'utilisation de ces QTLs sur le germoplasme canadien sera discutée. Selon nos résultats, la sélection *in vitro* et assistée à l'aide de marqueurs moléculaires offrent ensemble un moyen plus rapide et efficace de développer des cultivars de blé résistant à la fusariose de l'épi.

Mots clés : blé, *Fusariose* de l'épi du blé, sélection *in vitro*, mycotoxine, haploïdes doublés, désoxynivalénol, marqueur moléculaire

Poster 7.

Screening of spring wheat lines for genetic resistance to *Fusarium* head blight

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The identification of various potential resistance sources for wheat *Fusarium* head blight (FHB) constitute a very important step in the breeding process, in order to develop resistant cultivars with good agronomic and quality characteristics. The objective of this study was to evaluate the FHB resistance level of a collection of spring wheat lines, together with Frontana, Sumai3 and their derivatives. These lines have been evaluated for FHB resistance using point inoculation method in replicated greenhouse trials, and using molecular markers linked to FHB resistance QTLs on chromosomes 3A, 3BS, 5A and 6BS identified in wheat cultivars Frontana and Sumai3. Also, mature seeds from inoculated spikes were used to evaluate deoxynivalenol concentration using the ELISA method at Ottawa service lab. Wheat lines CM82036 and HY644 were performing as well as Sumai3 to the inoculation test, and shown the lowest DON content in the series of lines evaluated. Line FE1 got also a significantly low DON concentration. The following wheat lines FE1, FE17, AC Reed, and AC Crystal carry the same QTL 3A as Frontana, and CM82036, 93FHB37 and HY644 carry the 3BS QTL from Sumai 3. None of the lines tested carried QTLs 5A and 6BS.

Keywords: wheat, *Fusarium* head blight, deoxynivalenol, germplasm, molecular markers

Evaluation de lignées de blé de printemps pour leur résistance génétique à la fusariose de l'épi

L'identification de diverses sources potentielles de résistance à la fusariose de l'épi du blé représente une étape importante dans le processus d'amélioration des plantes, afin de développer de nouveaux cultivars avec de bonnes caractéristiques agronomiques et la qualité. L'objectif de cette étude était d'évaluer la résistance à la fusariose de l'épi chez une collection de lignées de blé de printemps, incluant Frontana, Sumai 3 et leurs dérivés. Ces lignées ont été évaluées en serre pour leur résistance à la fusariose de l'épi en utilisant la méthode d'injection, et à l'aide de marqueurs moléculaires pour les QTLs sur les chromosomes 3A, 3BS, 5A et 6BS identifiés chez les blés Frontana et Sumai 3. Les grains matures des épis inoculés ont été utilisé pour déterminer la concentration de désoxynivalénol (DON) par la méthode ELISA au laboratoire de service d'Ottawa. Les blés CM82036 et HY644 étaient aussi résistant que Sumai 3 lors du test d'inoculation artificielle et ont présenté les concentrations de DON les plus faible. La lignée FE1 présentait aussi une très faible teneur en DON. Les lignées suivantes FE1, FE17, AC Reed et AC Crystal portent le QTL 3A de Frontana, et les lignées CM82036, 93FHB37 et HY644 portent le QTL 3BS de Sumai 3. Aucune lignée testée ne portait les QTLs 5A et 6BS.

Mots clés : blé, *Fusariose* de l'épi du blé, désoxynivalénol, germoplasme, marqueur moléculaire

Poster 8.

High resolution profiling of wheat genes differentially expressed in response to *Fusarium graminearum* infection.

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Fusarium head blight (FHB) of wheat caused by *Fusarium graminearum* (Schw.) affects wheat production worldwide reducing yield and quality. Genome-wide expression profiling of genes altered during host-pathogen interactions may improve our understanding of the mechanism(s) underlying resistance to FHB. A cDNA biochip representing 5664 ESTs, derived from a suppression subtractive hybridization library of wheat-*F. graminearum* interactions, was used for tissue-specific profiling of differentially-expressed genes in response to *F. graminearum* infection. The 93FHB37 wheat line carrying three major resistance QTLs mapped to chromosomes 3BS, 6BS and 5AL was used for the study. Inoculated wheat spikes were dissected into tissues: glume, lemma, palea, ovary, anther and rachis. The monitoring of genes in specific tissues avoided the averaging of expression data that occurs when using an entire spike as a biological sample. Hybridizations were completed using 30 arrays including 5 independent hybridizations for each tissue. Significant analysis of microarrays (SAM) resulted in the identification of transcripts encoding defense and stress related proteins, components of the ethylene and the phenylpropanoid pathways and a member of WRKY transcription factor family. Analysis of variance revealed that about 37% of genes responding to *F. graminearum* showed a significantly different expression pattern among separate floral tissues.

Poster 9.

Stress-directed selection identifies lines of spring wheat with enhanced resistance to *Fusarium* head blight and other diseases.

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A protocol of ‘Stress-Directed Selection’ (SDS), an iterative approach in which populations are subjected to repeated rounds of multiple disease pressures and combined resistances selected) identified spring wheat populations with improved FHB resistance. This approach has already succeeded in identifying lines with superior FHB resistance when one parent of the cross was a known FHB-resistance source. This work examines the application of SDS to populations derived from crosses of elite, FHB-susceptible parents. BC₁F_n progeny of the cross cv ‘Superb’²/ ‘CO960293’ (spring wheat, winter wheat, respectively) were subjected to SDS and selected under FHB pressure in alternating generations after BC₁ F₄. A small proportion of the lines showed unexpectedly good FHB resistance which was confirmed under controlled conditions. The progeny of these lines were subjected to SDS and about half the population was clearly FHB-resistant while the other half was as susceptible as the recurrent parent. Application of SDS to a selfing population of the breeding line c2652 (highly FHB-susceptible, but being examined as a possible new source of wheat streak mosaic virus resistance) identified lines with improved and apparently stably inherited FHB resistance.

Poster 10.

Inheritance of resistance to *Fusarium* head blight in a recombinant inbred lines barley population

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Fusarium head blight (FHB) is presently the most significant disease of barley in Canada. Understanding the inheritance of FHB resistance in barley is valuable in designing resistance breeding strategies. One hundred and forty seven F₅ RILs from the cross 2ND16092/TR360 were screened twice in the greenhouse at CDC, Saskatoon and in the Brandon FHB field screening nursery in 2004 and 2005. In the greenhouse, the population was screened using a spray inoculation technique with a single *Fusarium graminearum* isolate (FG99). FHB severity and DON accumulation in the population was numerically lower than the mid-parent value indicating slightly greater resistance than expected. There was a strong correlation ($r = 0.74-0.83$) between FHB rating and DON accumulation indicating that greenhouse selection of resistant lines based on FHB severity may be feasible. In the field, the population was screened using the grain spawn method with a mixture of three *F. graminearum* isolates. Population distribution for FHB severity and DON accumulation was nearly continuous suggesting resistance is quantitatively inherited. A few lines showed resistance to FHB and DON accumulation in all the tests indicating that FHB resistant lines with acceptable DON accumulation could be developed but it is a slow, expensive process.

Poster 11.

Improvement of *Fusarium* head blight resistant winter wheat in Hokkaido, Japan.

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Fusarium Head Blight (FHB) is the most serious disease of wheat in Hokkaido, the northern part of Japan. The pathogens involved in FHB produce mycotoxins. FHB resistant resource of Asian, South American and European origins are well known, but there are few FHB resistant winter wheat. To improve FHB resistance of winter wheat, we crossed 'Sumai 3' or 'Saikai 165' (Sumai 3/Saikai 144: raised by National Agricultural Research Center for Kyushu, Japan) of a parent to introduce high FHB resistance to winter wheat cultivars of the Hokkaido district. We selected FHB resistant lines with irrigated and inoculated nurseries. Selected lines indicated the same FHB resistance as 'Saikai 165', and FHB resistance of a few selected lines exceeded that of 'Saikai 165'. The levels of DON content among selected lines were very low compared with cultivars in Hokkaido. Sprouting tolerance was also introduced in these FHB resistant lines, and it became easy to use as crossing parent to introduce FHB resistance, but they needed to be improved for snow mold resistance, yield and quality. The level of FHB resistance among the progeny strain which crossed >Sumai 3' with commercial cultivar 'Kitamoe' ranged from high resistant to sensitive.

Poster 12.

Development of a potential technology for high throughput screening of quantitative resistance in wheat cultivars against *Fusarium* head blight

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Genetic approach is considered to be an effective and promising way to manage the *Fusarium* head blight (FHB) of wheat. Development of high throughput screening tools to discriminate levels of resistance is one of the highest priorities in the FHB resistance breeding programs. Metabolic profiles were developed for six wheat cultivars varying in resistance against FHB, each inoculated with *Fusarium graminearum* conidial suspension or water. The disease severity was related to metabolic profiles in the process to develop a technology being able to discriminate the levels of resistance among cultivars.

Univariate ANOVA of the disease severity (AUDPC) classified the six cultivars into four resistance groups, in decreasing order of resistance: A) AW488, Wangshuibai, Nobeoka Bouzu; B) BRS177; C) Frontana; and D) CEP24. A canonical discriminant analysis of metabolic profiles/fingerprints based on abundances of 52 metabolites or abundances of 560 mass ions (40-600 m/z), each identified 4 canonical variables, explained all the variances among cultivars. A scatter plot of 3 CANs and also a cladogram following cluster analysis of the 4 CANs both produced four resistance groupings, similar to those based on disease severity. The individuals of highly resistant group (A) clustered close together. Water or pathogen inoculated individuals grouped together in separate clusters.

Poster 13.

Pyramiding genes for *Fusarium* head blight (FHB) resistance in winter wheat-RCATL33 and the future strategies

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Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) is an important disease of wheat. The disease reduces yield and quality, while *F. graminearum* produces mycotoxin deoxynivalenol (DON) in the grain. Different types of FHB resistance have been reported in wheat and one type of resistance alone is not sufficient to prevent severe FHB epidemics. The objective of this study was to pyramid FHB resistance genes from different sources and to develop a line of winter wheat with increased overall FHB resistance. We successfully developed winter wheat line RCATL33 (Ruby/Frontana#1//AC Ron/3/25R18/AC Ron) with combined FHB resistance from Sumai 3 (resistance to spread of symptoms within the spike) and FHB resistance from Frontana (resistance to initial infection). RCATL33 has a low level of symptom expression plus lower DON content. Genetic analysis indicates that RCATL33 contains Frontana alleles on chromosome 3A and Sumai 3 alleles on chromosome 3BS. The diagnostic band sizes for markers *barc 45* (3A), *gwm5* (3A), *gwm533* (3BS), *barc147* (3BS) and *gwm 493* (3BS) are 200 bp, 196 bp, 160 bp, 123 bp and 213 bp, respectively. Future work will include crossing RCATL33 with other unrelated sources of FHB resistance to further pyramid FHB genes from different sources.

Poster 14.

Ontario winter wheat performance trial - an index that account for *Fusarium* head blight symptoms and deoxynivalenol level jointly

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Fusarium head blight (FHB) index (%) and deoxynivalenol (DON) level from FHB inoculated Ontario Winter Wheat Performance trial (OWWPT) in 2004 was used in this study. The objectives were to develop an index for each variety that will account for FHB symptoms and DON level jointly, and then to group the varieties to susceptibility classes. Individual location was removed from the data set, and combined ranking of FHB index and DON level was applied. Although DON and FHB were essentially independent, the index that included them both was correlated to both of them, and therefore represents their joint effects. The varieties were grouped as follows: 1 = >1SD below mean (moderately resistant), 2 = <1SD below mean (moderately susceptible), 3 = <1SD above mean (susceptible), 4 = >1SD above mean (highly susceptible). In 2005 OWWPT trial, 5 varieties were classed as moderately resistant, 10 moderately susceptible, 10 susceptible and 5 highly susceptible. When applied to four year=s of FHB data, the index from 2004 also appears to be significant and robust ($r = 0.87$), therefore is useful to farmers in making variety selection decisions. A progress is being made in breeding and selection for FHB resistance in Ontario winter wheat, but there are still lots of room for improvements.

Poster 15.

Molecular breeding for improved *Fusarium* head blight resistance in wheat in Iran

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Molecular breeding for improved FHB resistance in wheat in Iran was started in 2002 by participating in scientific co-operation projects between Iran and Austria. The main objective of this work was to identify molecular markers linked to FHB resistance for marker assisted backcross (MAB) breeding with the aim to develop locally adapted wheat cultivars with improved *Fusarium* resistance in much shorter time than by conventional breeding. Different types of populations segregating and DNA markers (SSR, AFLP and RGA) were used for QTL mapping of FHB resistance. The preliminary QTL analyses results showed one consistent QTL for FHB resistance was identified on 3BS chromosome in both 'Wangshuibai' and 'Sumai-3' populations segregating. Three consistent QTLs for FHB resistance on chromosomes 1BL, 3AL and 7AS were detected on 'Frontana' populations segregating. Strong emphasis was placed on mapping candidate genes (*RPL3* and *PDR5*-like) and ESTs putatively involved in FHB resistance. The preliminary single marker analysis indicated that one gene-specific marker for *PDR5*-like genes had a significant negative effect on FHB severity in 'Wangshuibai' derived populations. Current work is transferring individual detected QTLs and markers associated with FHB resistance into local adapted cultivars. Furthermore, these markers will be used to test if there are any differences among the local FHB resistance Iranian sources with the widely used Chinese and Brazilian sources for the markers/genes linked to FHB resistance. Other works in progress are including identifying specific FHB infection responsive genes/proteins using transcriptome analysis (cDNA-AFLP) and two-dimensional gel electrophoresis (2D-GE) in our developed RILs and NILs.

Keywords: *Fusarium* head blight, wheat, QTL, candidate genes

Poster 16.

The effect of general field selection on wheat microsatellite allele frequencies at FHB resistance QTLs.

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The development of FHB resistant cultivars is an important objective of Canadian common wheat breeding programs. The objective of this study was to determine whether selection for agronomic and disease response traits affected microsatellite allele frequencies at FHB resistance quantitative trait loci (QTLs). FHB resistance QTLs, derived from Nyubai, Sumai 3, and Wuhan 1, were backcrossed into three elite western Canadian spring wheat backgrounds, 98B69-L47, BW301, and Kanata, using marker-assisted selection. Eight hundred and one doubled haploid (DH) lines were produced from all possible cross combinations among BC₂F₂ lines derived from these three backgrounds. The DH lines were selected for plant type, plant height, lodging, and time to maturity in New Zealand, and response to leaf rust, stem rust, common bunt, and FHB in nurseries near Swift Current, SK., and Carman, MB. All DH lines were analyzed with microsatellite markers at FHB resistance QTLs on chromosomes 2D, 3BS, 3BSc, 4B, and 5AS. Microsatellite allele frequency changes were not significant at most loci. Selection resulted in a moderate but significant reduction in Nyubai and Sumai 3 alleles at 5AS microsatellite loci. The Wuhan 1 allele at *Xgwm608-2D* was fixed in one population. In general, selection for general field performance and response to leaf and stem rust and common bunt did not adversely affect variation at FHB resistance QTLs in the populations evaluated, except for 5AS.

Poster 17.

The evaluation of FHB resistance QTLs introgressed into elite Canadian common wheat germplasm.

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The effects of *Fusarium* head blight (FHB) resistance quantitative trait loci (QTLs), from Nyubai, Sumai 3, and Wuhan 1, were evaluated in three elite Canadian spring wheat backgrounds, 98B69-L47 (QTLs: 3BS, 3BSc, 4B), BW301 (QTLs: 2D, 3BS, 5AS), and Kanata (QTLs: 3BSc, 5AS). Microsatellite loci, linked to these QTLs, were analyzed to identify homozygous BC₂F₂ individuals. BC₂F₃ and BC₂F₄ lines were evaluated for anthesis date, plant height, FHB incidence, FHB severity, FHB index, *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) content in an Ottawa FHB nursery in 2004 and 2005, respectively. In the 98B69-L47 population, the 4B QTL from Wuhan 1 had the largest impact and significantly reduced FHB in 2004. However, this QTL was also significantly associated with increased plant height in both years, which may effect its deployment. In the BW301 population, the 2D QTL from Wuhan 1 decreased FHB in both years while the 5AS QTL from Nyubai decreased FHB in 2004. In the Kanata population, the 3BSc QTL from Sumai 3 was more effective than the 5AS QTL from Sumai 3. The Nyubai 3BS QTL did not have a major effect on FHB in these tests. Overall, there was a general reduction in FHB symptoms as FHB resistance QTLs were introduced into an elite genetic background.

Poster 18.

Progress towards development of FHB resistant barley in the Crop Development Centre barley improvement program

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The Crop Development Centre (CDC) barley improvement program has participated in the collaborative AAFC, Brandon, MB. *Fusarium* Head Blight (FHB) screening nursery since its inception in 2000. Screening of several thousand CDC lines from 2001 to 2005 identified 9 hulled selections consistently demonstrating resistance to DON accumulation comparable or better than the resistant check CI4196. Two of these lines, 2ND16092 (from North Dakota State University) and HDE84194-622-1 (an accession from Shanghai Academy) were used as parents (initial FHB project crosses) in the CDC program. One hundred fourteen selections from 7 populations with 2ND16092 as a parent and 65 selections from 2 populations with HDE84194-622-1 as a parent were tested in 2005 for their FHB response and in Preliminary yield tests. SB00106 (TR04378), an agronomically promising line, showed low DON levels in the 2003 and 2004 North American Barley Scab Evaluation Nursery and was entered in the 2004 and 2005 Western Two Row Coop Test. It replaced CI4196 as the resistant check in the 2005 Brandon FHB nursery. In general, hulless lines showed lower DON accumulation and 30 have been selected with consistently lower FHB and DON accumulation over years and locations. These hulled and hulless selections are being used as parents to improve FHB resistance. A high portion of waxy starch hulless lines have shown resistance to FHB and DON accumulation in the greenhouse and field and are being further evaluated. Finally, testing of wheat-barley addition lines in the greenhouse indicated that BAL 7 (5H) had considerably lower FHB severity and DON accumulation than their highly susceptible wheat parent Chinese Spring.

Poster 19.

Breeding for resistance to *Fusarium* head blight in barley using 'exotic' sources of resistance

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Genetic resistance is the most desirable long-term method of controlling *Fusarium* head blight (FHB) in barley. Although few highly resistant barley lines have been found, several lines from various 'exotic' sources have repeatedly shown reduced disease symptoms and low deoxynivalenol (DON) accumulation. A number of these resistance

sources, including AC Sterling, Harbin, Morrison, Svanhals, Zhedar #1 and Chevron, have been used as parents by the two-row malting barley breeding program at Agriculture and Agri-Food Canada, Brandon, MB. Populations developed from crosses involving these resistance sources were evaluated over several years in an irrigated FHB nursery at Brandon, and further tested to confirm stability of resistance in subsidiary FHB nurseries at Charlottetown, PE, Ottawa, ON, Glenlea, MB and Portage la Prairie, MB. Fourteen lines consistently showing low DON accumulation were selected from yield trials. Half of these were from one doubled haploid population (pedigree: Harbin/TR253//TR253) which also had acceptable agronomic traits. TR04281, TR04282 and TR04283 were the first lines to enter the Western Cooperative Two-row Barley Registration Test in 2004, followed by TR05285, TR05286 and TR05287 in 2005. Even if none of these lines are released as new cultivars, they will serve as valuable germplasm for further crossing.

Amélioration de la résistance à la *fusariose* chez l'orge en utilisant des sources de résistance 'exotiques'

La résistance génétique est la méthode à long terme la plus efficace de commander la *fusariose* des panicules (FP) dans l'orge. Bien que peu de lignes fortement résistantes d'orge aient été trouvées, plusieurs lignées de diverses sources 'exotiques' ont souvent des symptômes réduits de la maladie et une accumulation réduite de désoxynivalénol (DON). Un certain nombre de ces sources de résistance, y compris AC Sterling, Harbin, Morrison, Svanhals, Zhedar #1 et Chevron, ont été employées comme parents pour l'orge à malter de deux-rang par le programme de Agriculture et Agroalimentaire Canada, Brandon, MB. Des populations développées, à partir des croisements impliquant ces sources de résistance, ont été évaluées pendant plusieurs années dans une pépinière irriguée de FP à Brandon, et encore examinées pour confirmer la stabilité de la résistance dans les pépinières subsidiaires de FP à Charlottetown, IPE, à Ottawa, ON, à Glenlea, MB et à Portage la Prairie, MB. Quatorze lignées montrant uniformément l'accumulation réduite de DON ont été choisies parmi des épreuves de rendement. La moitié de ces dernières lignées étaient de la population haploïde doublée Harbin/TR253//TR253 qui a également eu des traits agronomiques acceptables. TR04281, TR04282 et TR04283 étaient les premières lignées incorporées dans l'essai coopératif de l'ouest pour l'enregistrement de l'orge à deux rangs en 2004, suivi de TR05285, de TR05286 et de TR05287 en 2005. Même si aucune de ces dernières lignées ne deviennent de nouveaux cultivars, elles serviront de germoplasme très utile aux croisements futurs.

Poster 20.

A study on the feasibility of pyramiding FHB resistance genes with other resistance and agronomic traits

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Plant traits tend to be correlated with each other. For example FHB reaction appears to be influenced to some extent by plant morphology, root health, lodging, earliness, manganese uptake efficiency, and many other traits. The quantitative nature of FHB resistance has become accepted as a fact of life. The wisdom of the pursuit complex goals, however, has received slower acceptance, despite evidence from quantitative genetic studies showing that this leads to better selection efficiency. Part of our germplasm development approaches focused on better efficiency in the pursuit of complex goals. Three to five stresses, chosen among FHB, BYDV, drought, water logging, mineral deficient soils, rusts, mildew, root rot, were used simultaneously and often directly on F₁ plants. Lodging, biomass production ability, grain hardness, hectolitre weight, and morphological traits were also used. Through the production of 15000 F₁ (most of them 4-parent, i.e. F₁/F₁) and elimination of most of them by complex, severe, controlled stresses, a few wheat lines were obtained that can resist most of the quoted biotic and abiotic stresses while also having valuable agronomic traits. The rate of reject was 99% or more. The best germplasm obtained offers many resistance factors with fewer drawbacks. A germplasm release should follow.

Poster 21.

Associations between anther extrusion and *Fusarium* head blight in European wheat

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Anthers of cereals are involved in the infection process of *Fusarium spp.* which could be explained by stimulation of fungal growth by choline etc. Some wheat breeders in Europe have found a positive correlation between the degree of anther extrusion (AE) and the level of resistance to *Fusarium* Head Blight in wheat. In 2005 we tested 107 varieties of winter wheat from different breeders in Northern Europe with spray inoculation by *F. culmorum*, and a correlation of - 0.61 was found between FHB and AE. In the mapping population of 93 DH lines from Arina x NK93604 we got a correlation of - 0.66 and -0.52 between AE in 2005 and FHB 2001- 2003, resp. DON 2002. The heritability of AE was high (0.90), accordingly a correlated response for FHB is expected. Results from Composite Interval Mapping using 624 AFLP, DArT or SSR markers showed QTL's for AE explaining above 10% on 1AL, 2DS, 6AS and 7AL, two of them were from Arina and two from NK93604. The factors on 1AL and 7AL were located 48 resp. 32 cM from FHB QTL's, one case in coupling, another in repulsion. The presence of few genetic factors for AE is in correspondance with the literature.

Relations entre l'extrusion d'anthère et la fusariose

Il y a un lien entre les infections de la *fusariose* et les anthères de certaines céréales et qui pourrait être du à la stimulation de la croissance d'un champignon provoqué par la choline etc. Des sélectionneurs de blé Européens ont trouvé une corrélation entre le degré d'extrusion des anthères (EA) et le niveau de résistance à la fusariose de l'épi chez le blé (FHB). En 2005 on a testé 107 variétés de blé d'hiver provenant de différents sélectionneurs d'Europe du Nord; après inoculation par aspersion du *F. culmorum* on a trouvé une corrélation négative de -0,60 entre FHB et EA. Dans une population cartographie de 93 lignées haploïides doubleés à partir d'Arina x NK93604 on a obtenu une corrélation de - 0,66 et - 0,52 entre EA en 2005 et FHB entre 2001-2003, resp. DON 2002. L'héritabilité des EA était élevée (0,90) et on peut donc s'attendre à une corrélation analogue pour FHB. Les résultats du CIM cartographie en utilisant les marqueurs 624 AFLP, DART ou SSR ont fait ressortir des QLT's pour EA expliquant ainsi une augmentation de 10% sur 1 AL, 2DS, 6AS et 7AL, deux d'entre eux provenant d'Arina et deux autres de NK93604. Les facteurs sur 1 AL et 7AL ont été localisés sur 48 resp. 32 cM de FHB QLT's, dans un cas par association, dans un autre par ségrégation. La présence de quelques facteurs génétiques pour les EA est en corrélation avec les documents actuels.

Poster 22.

New wheat cultivars with improved resistance to FHB for Eastern Canada

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The reaction of wheat lines to *Fusarium* head blight can vary quite a lot and is dependent on flowering dates and daily weather conditions. For this reason, it takes many years and test sites to properly assess the FHB reaction of a candidate line or cultivar. Moreover, the field symptoms are not very good predictors of toxin levels in the harvest. The correlations can range from poor to good depending on years and sites. Data will be presented to show the relationship between field symptoms and toxin content and to describe some new promising cultivars.

Nouvelles variétés de blé avec résistance améliorée contre la fusariose pour l'est du Canada

La réaction de lignées de blé à la *fusariose* de l'épi peut varier beaucoup et dépend de la date de floraison et des conditions météorologiques. Pour cette raison, la mesure de la réaction d'une lignée ou d'un cultivar à la fusariose demande plusieurs années et plusieurs sites de tests. En plus, les symptômes visuels ne sont pas bien corrélés aux niveaux de toxine à la récolte. Les corrélations vont de pauvres à bonnes dépendant des années et des sites. Nous présentons des données pour montrer cette corrélation entre les symptômes visuels et la concentration de toxines, et pour décrire quelques nouveaux cultivars prometteurs.

Poster 23.

A new source of resistance to *Fusarium* head blight in spring wheat

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a very destructive disease of spring wheat in Ontario. Sources of resistance are limited and hinder the development of improved disease resistant cultivars. New sources of resistance must be identified and exploited. The objective of this study was to transfer FHB resistance from *Triticum timopheevii* to spring wheat *Triticum aestivum*. Crocus spring wheat was crossed to *T. timopheevii*, PI343447, in the greenhouse in 1999. Crocus is a line with the genetic background of cv. Columbus plus three crossability genes *kr1*, *kr2* and *kr3*. Following a backcross with Crocus, 535 BC₁F₇ lines were developed, using single seed descent (SSD). One hundred lines were selected, based on plant fertility and agronomic traits, and evaluated for reaction to FHB in the greenhouse for two seasons. One line, TC 67, had good resistance to FHB, comparable to that of Sumai 3, based on point inoculation. This line was also evaluated in a 4-replication FHB nursery in 2003 and 2004. FHB incidence and severity of TC 67 were much lower than those of the HY 644 check and its DON level was comparable to that of Sumai 3. TC 67 was crossed with AC Brio and an F₆ population of 235 lines was developed through SSD. These lines are being phenotyped for FHB reaction and thus the genes for resistance to FHB will be mapped.

Keywords: *Fusarium* head blight, *Triticum aestivum*, *Triticum timopheevii*

Poster 24.

Development of a PCR-DGGE molecular method for *Gibberella/Fusarium* identification and monitoring *in situ*

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A total of 14 *Gibberella/Fusarium* species were associated with asparagus production decline (~40%) in Quebec. The fast identification of the principal *Gibberella/Fusarium* species is essential to determine the composition of the complex of pathogenic species in order to establish efficient control strategies. Thus, the specific primers targeting the EF-1 alfa gene and a culture- independent DGGE method to assess *Gibberella/Fusarium*, both in pure culture and in environmental samples, were developed. The application of the PCR-DGGE method makes possible to rapidly detect the main pathogenic species (*F. proliferatum* and *F. oxysporum*) present in the field but does not allow distinguishing between virulent and avirulent strains. On the other hand, the results suggested that the highly pathogenic species in asparagus, *Fusarium proliferatum*, may be favoured with a previous maize crop, as the disease level is significantly increased in this case. The collaborative effort between the University of Saskatchewan, Agriculture and Agri-Food Canada and the University of Montreal research groups was established aiming to develop more accurate methods to detect and identify the *Gibberella/Fusarium* species diversity that could be found in renewable plant resources (agriculture and forestry).

Poster 25.

Aggressiveness comparison of two *Fusarium graminearum* isolates-mixes according to their deoxynivalenol producing ability and their effect on the selection of resistant barley to *Fusarium* head blight

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The screening of barley genotypes for their resistance to *Fusarium* head blight (FHB) in regional field nurseries is one of the final steps for selection. Field nurseries are exposed to different inoculum sources from different *Fusarium graminearum* isolates. The resulting selection process may lead to broader use of the selected genotypes with FHB resistance, because a selection process based on one particular inoculum may be valid on fewer isolates. Therefore, I have tested two inoculums (isolates-mixes) currently used in FHB breeding nurseries in Ontario and in Québec in two side by site experiments in Ottawa to verify their infection capacity and their DON producing ability. Results show that, over a two-year period, both inoculums were valid for the selection of the same genotypes for their resistance to FHB according to visual symptoms. A greater aggressiveness of the Ontario isolates was observed with 62% more DON in the genotypes compared to Québec isolates. The aggressiveness of isolates-mixes was greater in 2004, a more favourable year for FHB development contributing to more instability in genotypes selection for resistance to DON accumulation. Therefore, it may be possible that more test-years are required to achieve stability for FHB resistance if more aggressive isolates are used.

Comparaison de l'agressivité de deux mélanges d'isolats de *Fusarium graminearum* et leur capacité à produire le désoxynivalénol et leur effet sur la sélection de la résistance à la fusariose de l'épi chez l'orge

Le tamisage des lignées d'orge à travers le réseau des pépinières régionales de fusariose de l'épi constitue une des dernières étapes de sélection pour la résistance. Les pépinières sont exposées à différentes sources d'inoculum provenant d'isolats différents de *Fusarium graminearum*. La sélection qui en résulte devrait servir à une utilisation plus extensive des génotypes résistants, car celle basée sur une seule source d'inoculum pourrait n'être valable que pour une exposition à un nombre limité d'isolats. J'ai donc utilisé deux inoculums (mélanges d'isolats) couramment utilisés dans les pépinières au Québec et en Ontario dans deux essais adjacents réalisés à Ottawa, afin d'évaluer leur capacité à infecter et à induire la production de DON. Les résultats obtenus sur une période de deux ans démontrent que les deux sources d'inoculum sont efficaces pour la sélection des mêmes génotypes plus résistants selon les symptômes visuels de fusariose. Cependant, l'agressivité des isolats utilisés en Ontario en fonction du DON a été 62% supérieure comparativement à ceux du Québec. Cette différence a occasionné plus de variation dans le choix des génotypes résistants. L'agressivité des mélanges d'isolats s'est accrue en 2004, une année plus favorable au développement de la fusariose. Une instabilité de la résistance au DON s'y est manifestée. Il est donc possible que plus

d'années d'essais soient requises pour atteindre une meilleure stabilité de la résistance si des isolats plus agressifs sont utilisés.

SESSION II - BIOTECHNOLOGY

Impact of Trichothecenes on Disease

Impact des trichothécènes sur la pathogénèse

François Eudes, Agriculture and Agri-Food Canada

Exploring the Life Cycle of *Fusarium graminearum* using Genomics

Frances Trail, Michigan State University

Impact of Trichothecenes on Disease

François Eudes

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In Canada, maize, wheat and barley diseases caused by *Fusarium graminearum*, have re-emerged in the 80-90s. This saprophyte fungus infects roots, young tissues and flowers at anthesis stage and affects the crops in two ways: it reduces the yield and affects the quality of the grains. This fungus produces mycotoxins that represent a food and feed safety issue. Two main classes of mycotoxins are produced by *Fusarium graminearum*, the trichothecenes and zearalenone. Trichothecenes are protein synthesis inhibitors, and also phytotoxins. Independent studies using isogenic *Fusarium graminearum* strains, producing and non-producing trichothecene, revealed that trichothecenes are a factor of virulence for the spreading of the fungus in small grain cereals. However, trichothecenes are not a factor of virulence for the initial infection. These experiments provided evidence for a more significant contribution of *F. graminearum* trichothecenes and corresponding resistance mechanisms in small grain cereal disease than initially thought and relative to FHB resistance to initial infection and to spread. Based on this information, early screening methods have been developed and applied to wheat and barley in Canada with various levels of success. Latest results in the effort to introgress FHB resistance in wheat and barley germplasm and cultivars in Canada will be presented.

Keywords: trichothecenes, resistance mechanisms, cereals, *Fusarium graminearum*

Impact des trichothécènes sur la pathogénèse

Au Canada, *Fusarium graminearum* est réapparu dans les années 80-90, causant des maladies chez le maïs, le blé et l'orge. Ce saprophyte infecte les racines, les jeunes tissus et les fleurs au stade anthèse, affectant entraînant une perte de rendement et de qualité. Ce champignon produit des mycotoxines qui représentent un risque alimentaire. Deux familles principales de mycotoxines sont produites par *F. graminearum*, les trichothécènes et les zéaralénones. Les trichothécènes sont des inhibiteurs de la synthèse protéique et des phytotoxines. Des études indépendantes, utilisant des souches isogéniques de *F. graminearum*, productrice et non productrices de trichothécènes, ont démontré que ces toxines sont des facteurs de virulence lors de la progression du champignon dans les tissus, mais pas dans la phase initiale d'infection. Ces expériences ont fait la démonstration d'un rôle plus important qu'anticipé des trichothécènes de *F. graminearum* et des mécanismes de résistance correspondant, en particulier par rapport aux deux résistances traditionnellement décrites. Au Canada, des méthodes de sélection précoces ont été élaborées et appliquées chez le blé et l'orge avec plus ou moins de succès. Les résultats récents de ces tentatives d'introduire la résistance dans les germoplasmes de blé et d'orge ainsi que chez les cultivars canadiens seront présentés.

Mots clés : trichothécènes, mécanismes de résistance, céréales, *Fusarium graminearum*

Exploring the Life Cycle of *Fusarium graminearum* using Genomics

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The genome of *Fusarium graminearum* has been sequenced and genome-based microarray chips are now available. These tremendous resources provide an opportunity to study the life cycle of this important plant pathogen at a level not previously within reach. We have begun to elucidate the gene expression shifts that accompany spore production *in vitro* and in the host plant. These studies have revealed that production of ascospores is associated with specific host cells. During host colonization, the fungus also produces mycotoxins, including fusarin, zearalenone and aurofusarin, which make the grain unfit for human and animal consumption. These polyketide mycotoxins are produced by Polyketide Synthases (PKS). We used the genomic sequence to identify all the PKS genes in the genome. We then disrupted each gene individually and analyzed the mutants phenotypically. We have identified the genes responsible for biosynthesis of these mycotoxins. We continue to explore their role in the life cycle of this important pathogen. These studies provide new targets for control of this devastating pathogen. Elucidation of the developmental processes underlying the disease cycle and the response of the pathogen to the environment is an important step towards developing integrated control strategies.

SESSION II - BIOTECHNOLOGY

26.

New *in vitro* selection method for isolated microspore culture to improve *Fusarium* head blight resistance in barley

Nouvelle méthode de sélection *in vitro* pour culture de microspores isolées afin d'améliorer la résistance à la *fusariose* de l'orge

Banik, M.; Legge, W.G.; Tucker, J.R.; Therrien, M.C.; Tekauz, A.; Eudes, F.; Savard, M.E.; Rosnagel, B.G.

27.

Using genomic and proteomic tools to characterize the response of maize to *Fusarium graminearum*

Utilisation des outils de la génomique et de la protéomique pour la caractérisation de la réaction du maïs à *Fusarium graminearum*

Harris, L.; Anoop, V.; Johnston, A.; Schneiderman, D.; Saparno, A.; Balcerzak, M.; Kelso, S.; Sprott, D.; Gleddie, S.; Robert, L.; Tinker, N.; Ouellet, T.

28.

Comparison of barley seed proteomic profiles associated with *Fusarium* head blight reaction

Zantinge, J.; Kumar, K.; Xi, K.; Murray, A.; Johns, M.; Helm, J.H.

29.

Comparison of the response of Roblin, Wuhan and NuyBay to *Fusarium* attack using a genomic and proteomic approach

Comparaison de la réponse de Roblin, Wuhan et NuyBay lors d'une attaque par *Fusarium* en utilisant les approches génomique et protéomique

Ouellet, T.; Wang, L.; Schneiderman, D.; Balcerzak, M.; Tinker, N.; Harris, L.

30.

Development of a rapid PCR method to screen for the 3BS *Fusarium* head blight resistance QTL in Sumai-3

Matus-Cadiz, M.; Pozniak, C.; Hughes, G.; Hucl, P.

31.

Transgressive segregation for *Fusarium* head blight resistance in four durum wheat populations

Pozniak, C.J.

32.

Functional genomics and proteomics studies of *F. graminearum*

Études de génomique et de protéomique fonctionnelle de *F. graminearum*

Saparno, A.; Harris, L.; Alexander, N.; Blackwell, B.; McCormick, S.;

Desjardins, A.; Anoop, V.; Schneiderman, D.; Kelso, S.; Sprott, D.; Robert, L.; Tinker, N.; Gleddie, S.; Ouellet, T.

33.

Fine mapping *Fhb1*, a major gene controlling *Fusarium* head blight resistance in bread wheat (*Triticum aestivum* L.)

Cuthbert, P.A.; Somers, D.J.; Thomas, J.; Cloutier, S.; Brulé-Babel, A.

34.

Qualitative mapping of the *Fusarium* head blight resistance gene on chromosome 6B in bread wheat (*Triticum aestivum* L.)

Cuthbert, P.A.; Somers, D.J.; Brulé-Babel, A.; Gilbert, J.

35.

Comparison of marker-assisted and conventional selection for resistance to *Fusarium* head blight in a backcross breeding program in wheat.

Cao, W.; Fedak, G.; Somers, D.; McCartney, C.; Xue, A.; Savard, M.

36.

Monitoring of *Fusarium* species in soybean roots using DNA arrays and reverse dot-blot hybridization

Dépistage des espèces de *Fusarium* présentes dans les racines de soja à l'aide de la technique d'hybridation avec les macropuces à ADN

Barasubiye, T.; Seifert, K.A.; Tenuta, A.; Rioux, S.; Anderson, T.; Welacky, T.W.; Lévesque, C.A.

37.

A systems approach to *Fusarium graminearum* biology

Nasmith, C.; Wang, L.; Ouellet, T.; Harris L.; Subramaniam, G.

Poster 26.

New *in vitro* selection method for isolated microspore culture to improve *Fusarium* head blight resistance in barley

Banik, M.¹; Legge, W.G.¹; Tucker, J.R.¹; Therrien, M.C.¹; Tekauz, A.²; Eudes, F.³; Savard, M.E.⁴; Rossnagel, B.G.⁵

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Ottawa, ON.; ⁵ Crop Development Centre, University of Saskatchewan, Saskatoon, SK.

An *in vitro* selection study was conducted to develop a more efficient method using mycotoxin in the isolated microspore culture system for doubled haploid production to select mycotoxin tolerant barley plants with improved *Fusarium* head blight (FHB) resistance. Since isolated microspores are typically cultured on solid media, a special 'bridge' was constructed to culture them in liquid media containing the selective agent. This provided all microspores with an equal amount of stress and prevented them from sinking. Four media were used to culture the microspores of several different barley F1's and cultivars. The media containing the basic macro and micro nutrients of FHG media plus 400 mg/l myo-inositol, 72 gm/l maltose, 1 mg/l 6-Benzylaminopurine (BAP), 6.67 mg/l Phenylacetic acid (PAA), 0.33 mg/l Indole-3-acetic acid (IAA), 3.33 mg/l silver nitrate and 50 g/l Ficoll gave the best response for embryo induction and green plant regeneration. This new method may be more efficient in enhancing FHB resistance in barley, as individual cells are more uniformly exposed to selective pressure from the mycotoxins of the pathogen.

Keywords: barley, *Fusarium* head blight, *in vitro* selection, microspore culture, mycotoxin, doubled haploids, deoxynivalenol

Nouvelle méthode de sélection *in vitro* pour culture de microspores isolées afin d'améliorer la résistance à la fusariose de l'orge

Une étude *in vitro* a été entreprise pour développer une méthode plus efficace de sélection précoce en utilisant des mycotoxines en culture de microspore isolée pour la production d'haploïdes doublés d'orge tolérantes aux mycotoxines et ayant une résistance accrue à la *Fusariose* de l'épi. Puisque la culture de microspores isolées se fait généralement sur des milieux solides, un 'pont' spécial a été construit pour les cultiver sur un milieu liquide contenant l'agent sélectif. Ceci permet d'exercer une pression de sélection uniforme sur les microspores et les maintiens à la surface du milieu. Quatre milieux ont été utilisés pour cultiver les microspores de plusieurs hybrides F1 ainsi que des cultivars. Les milieux contenant les macro et les micro éléments de base du milieu FHG plus 400 mg/l de myoinositol, 72 gm/l de maltose, 1 mg/l de 6-Benzylaminopurine (BAP), 6,67 mg/l d'acide phénylacétique (PAA), 0,33 mg/l d'acide Indole-3-acetic (IAA), 3,33 mg/l de nitrate d'argent et 50 g/l de Ficoll ont donné la meilleure réponse pour l'induction d'embryons et la régénération de plantes vertes. Cette nouvelle méthode pourrait être plus efficace en augmentant la résistance à la fusariose de l'épi chez l'orge, car les cellules sont plus uniformément exposées à la pression sélective des mycotoxines

de ce pathogène.

Mots clés : l'orge, la *fusariose* de l'épi du blé, la sélection *in vitro*, culture de microspore isolée, mycotoxine, haploïdes doublés, desoxynivalenol

Poster 27.

Using genomic and proteomic tools to characterize the response of maize to *Fusarium graminearum*

Harris, L.; Anoop, V.; Johnston, A.; Schneiderman, D.; Saparno, A.; Balcerzak, M.; Kelso, S.; Sprott, D.; Gleddie, S.; Robert, L.; Tinker, N.; Ouellet, T.
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We are studying maize gene and protein expression during the early infection of maize with *F. graminearum* (*Fg*) with the goal of understanding how resistant and susceptible plants respond to *Fusarium* attack. Gene expression profiling of kernel and silk tissues was performed using a 6.6K maize cDNA microarray, comparing mock-inoculated and *Fg*-inoculated tissues from susceptible and resistant inbreds during the initial 48 hours of infection. Simultaneously, proteomes of the same samples at 48 hours post-inoculation were analyzed using two-dimensional-gel-electrophoresis (2DGE) and isotope-coded-affinity-tagged systems (ICAT: labels cysteines; iTRAQ: labels amine groups) to assess the impact of *Fg* attack on protein expression and identify proteins associated with enhanced *Fg* susceptibility/resistance. Large-scale genomic and proteomic analyses have revealed the differential expression of several PR proteins, glucosyltransferases, oxidoreductases, methyltransferases, and lipoxygenases, belonging to various primary/secondary metabolism pathways. For example, we have observed the up-regulation of many isoprenoid biosynthetic pathways genes in infected susceptible and resistant maize tissues while in susceptible wheat, many of these gene homologs are not affected by *Fusarium* attack. Some isoprenoids synthesized in response to pathogen attack can act as signalling molecules or antimicrobial compounds (phytoalexins). Functional characterization of selected genes/proteins is underway to establish their roles in *Fg* resistance in maize.

Utilisation des outils de la génomique et de la protéomique pour la caractérisation de la réaction du maïs à *Fusarium graminearum*

Nous étudions l'expression génique et protéinique du maïs en début d'infection par *F. graminearum* (*Fg*) afin de comprendre comment les types de maïs résistants et susceptibles réagissent à une infection à *Fusarium*. Pour déterminer le profil de l'expression génique dans les tissus du grain et des soies, nous avons utilisé une micropuce d'ADNc de maïs de 6,6 K et comparé des tissus traités par inoculation simulée ou par inoculation de *Fg* provenant de lignées autofécondées susceptibles et résistantes durant les premières 48 heures de l'infection. En outre, 48 heures après l'inoculation, nous avons aussi analysé le protéome des mêmes échantillons par électrophorèse en gel bidimensionnelle avec marquage d'affinité isotopique (ICAT : cystéines; iTRAQ : groupements amines) pour évaluer l'impact de l'infection à *Fg* sur l'expression protéique et déterminer quelles protéines sont associées à l'augmentation de la susceptibilité ou de la résistance à *Fg*. Les analyses génomiques et protéomiques à grande échelle ont permis de constater l'expression différentielle de plusieurs protéines PR (PR : << pathogenesis related >>), de glucosyltransférases, d'oxydoréductases, de méthyltransférases et de lipoxygénases intervenant dans diverses voies métaboliques primaires ou secondaires. Par exemple, nous avons pu observer la stimulation de nombreux gènes des voies de biosynthèse d'isoprénoïdes dans les tissus de maïs susceptibles et résistants infectés,

tandis que chez le blé susceptible, bon nombre des gènes homologues ne sont pas touchés par une attaque de *Fusarium*. Certains des isoprénoïdes synthétisés en réaction à l'attaque d'un agent pathogène peuvent jouer le rôle de molécules de signalisation ou d'antimicrobien (phytoaléxines). Nous travaillons actuellement à la caractérisation fonctionnelle de certains des gènes et de certaines des protéines en vue de déterminer leur rôle dans la résistance à *Fg* chez le maïs.

Poster 28.

Comparison of barley seed proteomic profiles associated with *Fusarium* head blight reaction

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A preliminary proteomic study was conducted on mature barley seed from cultivars/lines previously shown to inhibit the growth of the fungus *Fusarium graminearum* Schwabe (teleomorph: *Gibberella Zeae* (Schw.) Petch) *in vitro* and have partial resistance to fusarium head blight (FHB) in the field. The acidic and water-soluble proteins in extracts of mature barley seed from six barley cultivars/lines, 3 susceptible ('AC Lacombe', 'Stander', CI4196) and 3 partially resistant ('Chevron', Penco/Chevron, I92130) to FHB *in vitro* were evaluated by using two-dimensional polyacrylamide gel electrophoresis (2DE). About 171 protein spots of pI 4-7, detected by colloidal Coomassie Brilliant Blue staining, were found to differ between the 6 cultivars/lines. A total of 22 spots were identified potentially associated to resistance or susceptibility to FHB and these spots were analyzed by MS/MS liquid chromatography. Database searches resulted in 19 protein identifications. The majority of spots appeared to contain various enzymes with functions relating to house keeping, however, one spot uniquely found only in resistant 'Chevron' and Penco/Chevron shared homology with a putative Z-type serpin of barley. Plant serpins are thought to inhibit exogenous proteinases capable of breaking down seed storage proteins and have emerged as a class of antifungal proteins that have potent activity against plant pathogens. In order to verify these results, we plan to continue this study on additional related barley lines.

Keywords: *Fusarium graminearum*, *Fusarium* head blight, *Hordeum vulgare*, serpins, protein.

Poster 29.

Comparison of the response of Roblin, Wuhan and NuyBay to *Fusarium* attack using a genomic and proteomic approach

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We have compared the molecular response of 4 spring wheat varieties to attack by *Fusarium graminearum*: Roblin (very susceptible), Wuhan 1 and Sumai 3 (type II resistances from Chinese source) and NuyBay (resistance from Japanese source). Wheat heads at mid-anthesis were point inoculated in two florets for each spikelet and all fully developed spikelets were inoculated on each head. Inoculated spikelets and rachis were samples after 0, 1, 2 and 4 days of infection. Two biological replicates were performed. Analysis for the spikelet samples will be presented. Northern analysis was performed on all samples of the 4 varieties to establish a reference base line using the following pathogenesis-related (PR) genes: peroxidase, PR1, 1,3 B-glucanase (PR2), chitinase (PR3), PR4, thaumatin-like (PR5), and phenylalanine lyase (PAL). In parallel, microarray hybridization experiments have been conducted with the wheat Affymetrix chip, comparing mock-inoculated and *Fg*-inoculated spikelets from Roblin, Wuhan and NuyBay. Proteomic analysis is also being conducted on 2-days infected samples from Roblin and Wuhan, using iTRAQ to do a 4-way comparison of protein expression between mock and *Fg*-inoculated spikelets from susceptible and resistant varieties. Many differences could be observed in the response of susceptible and resistant varieties to *Fg* infection and will be summarized in the presentation.

Comparaison de la réponse de Roblin, Wuhan et NuyBay lors d'une attaque par *Fusarium* en utilisant les approches génomique et protéomique

Nous avons comparé la réponse moléculaire de 4 variétés de blé de printemps lors d'une attaque par *Fusarium graminearum* : Roblin (très sensible), Wuhan I et Sumai 3 (résistances de type II provenant de Chine), et NuyBay (résistance provenant du Japon). Pour chaque épi de blé à mi-anthèse, deux fleurettes ont été inoculées pour chaque épillet et tous les épillets développés furent inoculés. Les épillets inoculés et les rachis de chaque épi ont été échantillonnés après 0, 1, 2 et 4 jours d'infection. Chaque expérience fut performée deux fois. L'analyse des échantillons d'épillets sera présentée. Une analyse par Northern fut faite sur les échantillons des 4 variétés pour établir une ligne de base de référence en utilisant les gènes associés à la pathogénèse suivant : peroxidase, PR1, 1,3 B-glucanase (PR2), chitinase (PR3), PR4, thaumatin-like (PR5), et phenylalanine lyase (PAL). En parallèle, des hybridations sur biopuces ont été faites en utilisant la puce de blé d'Affymetrix, comparant des épillets de Roblin, Wuhan et NuyBay inoculés à blanc ou avec *Fg*. Une analyse protéomique est aussi en cours sur des échantillons de Roblin et Wuhan infectés pour 2 jours. La méthode iTRAQ est utilisée pour faire une comparaison à quatre sens des protéines exprimées entre les épillets inoculés à blanc ou avec *Fg* chez des variétés sensible et résistante. Plusieurs différences ont été observées dans la réponse des variétés sensible et résistante à l'infection par *Fg* et seront résumées lors de la présentation.

Poster 30.

Development of a rapid PCR method to screen for the 3BS *Fusarium* head blight resistance QTL in Sumai-3

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Selection for *Fusarium* Head Blight (FHB) resistance is complicated by the effects of environment on disease development and phenotypic expression of resistance. However, the use of molecular markers to indirectly select for FHB resistance genes can be used to increase selection efficiency as markers are not influenced by environmental conditions. The QTL on chromosome 3BS flanked by SSR markers *Xgwm493* and *Xgwm533* is thought to carry a gene(s) with a major effect for FHB resistance in Sumai-3 and its derivatives. However, use of these markers requires screening on polyacrylamide gels, which is a rate limiting step for the large scale screening required by our breeding programs. Furthermore, a multiplex PCR approach is desirable to simultaneously select for both markers flanking the QTL region. The objective of this work was to simplify a previously published MAS method by developing a multiplex PCR protocol for *Xgwm493* and *Xgwm533* and subsequent separation of amplicons using 2% agarose gels. Using this modified method, we were able to distinguish the majority of resistant and susceptible spring and durum wheat parents routinely used in our crossing programs. Although this method is not recommended for classifying previously uncharacterized genotypes as possessing the Sumai-3 3BS QTL, it has allowed us to advance early generation lines that are, at minimum, carrying the major FHB QTL on 3BS. We are conducting further studies to develop multiplex conditions in combination with high resolution agarose gels to enhance selection for multiple FHB QTL from Sumai-3 and other sources.

Poster 31.

Transgressive segregation for *Fusarium* head blight resistance in four durum wheat populations

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The development of durum wheat (*T. turgidum* var. *durum*) cultivars with improved *Fusarium* Head Blight (FHB) resistance has been limited in part due to a lack of effective resistance genes. However, we previously identified four accessions of Emmer wheat (*T. turgidum* var. *dicoccum*) which consistently displayed improved Type II FHB resistance in both field and greenhouse studies. To develop populations for genetic analyses, each accession was backcrossed once to DT735. In 2005, 75 F_{2:3} families from each of the four populations were evaluated for resistance to FHB at Carman, MB. Each family, together with their parental lines and repeated rows of check cultivars, were grown in 2.5 m rows. Rows were inoculated with *Fusarium graminearum* at anthesis and again four days after the first inoculation date followed by mist irrigation to promote disease development. Approximately 21 days after inoculation, each family was rated for incidence and severity of FHB infection and a FHB index was calculated as a measure of resistance. Bidirectional transgressive segregation for FHB resistance was evident in all populations suggesting that the Emmer accessions and DT735 are contributing desirable FHB resistance genes to their progeny. Differences in the frequency of families with low FHB indices were noted among the four populations, but in all cases, F_{2:3} families were observed with FHB indices well below the most resistant parent of the cross population. These results suggest that improvements in FHB resistance in durum can be made by combining genes from different sources of FHB resistance.

Poster 32.

Functional genomics and proteomics studies of *F. graminearum*

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A 4.5K unigene *F. graminearum* (*Fg*) cDNA microarray produced at our institute has been used in time-course experiments to profile *Fg* gene expression during liquid culture conditions favouring trichothecene production (with concurrent metabolic profiling). In-depth analysis of microarray results (three biological replicates) has identified candidate genes that may play significant roles in *Fg* pathogenicity. The gene expression patterns of 19 genes were validated by Northern and/or Real-Time PCR analysis. Two biological replicates of these liquid cultures have been subjected to iTRAQ analysis (isotope-coded-affinity-tagged proteomics technology), identifying 330 and 944 *Fg* proteins, respectively, and providing quantitative protein expression data over the liquid culture time course. The microarray and iTRAQ data were compared to identify common genes/proteins. In particular, gene and protein expression of a novel *Fg* gene cluster are co-ordinately induced during trichothecene-producing liquid culture conditions. Disruption and add-back analysis of one gene within this gene cluster suggests it contributes to butenolide production.

Études de génomique et de protéomique fonctionnelle de *F. graminearum*

Nous avons utilisé une micropuce d'ADNc monogénique de *F. graminearum* (*Fg*) de 4,5 K produite chez nous pour des expériences sur l'évolution chronologique de l'expression génique chez *Fg* durant la culture dans un milieu liquide favorisant la production de trichothécène (avec l'établissement parallèle du profil métabolique). L'analyse approfondie des résultats des épreuves sur la micropuce (trois échantillons biologiques identiques) a permis d'identifier des gènes susceptibles de jouer un rôle important dans la pathogénicité de *Fg*. Nous avons validé le profil d'expression de 19 gènes par transfert Northern et/ou par PCR en temps réel. Nous avons analysé deux des échantillons biologiques de cultures en milieu liquide avec le système iTRAQ (technologie protéomique de marquage d'affinité isotopique), ce qui a permis d'identifier 330 et 944 protéines de *Fg*, respectivement, et nous a apporté des données quantitatives sur l'expression protéique durant le cours de la culture. Nous avons comparé les données obtenues par l'analyse avec la micropuce et le système iTRAQ pour repérer les gènes et les protéines que les deux échantillons avaient en commun. En particulier, dans le cas d'une nouvelle batterie de gènes de *Fg*, nous avons constaté que l'expression génique et protéique est déclenchée de façon coordonnée durant la culture dans un milieu favorisant la production de trichothécène. L'analyse de perturbations et de réintégrations de l'un des gènes de cette batterie indique qu'il pourrait contribuer à la production de buténolide.

Poster 33.

Fine mapping *Fhb1*, a major gene controlling *Fusarium* head blight resistance in bread wheat (*Triticum aestivum* L.)

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A major *Fusarium* head blight (FHB) resistance gene *Fhb1* was fine mapped on the distal segment of chromosome 3BS of spring wheat (*Triticum aestivum* L.) as a Mendelian factor. FHB resistant parents, Sumai 3 and Nyubai, were used as sources of this gene. Two mapping populations were developed to facilitate segregation of *Qfhs.ndsu-3BS* in either a fixed resistant (Sumai 3*5/Thatcher) (S/T) or fixed susceptible (HC374*3/98B69-L47) (HC/98) genetic background (HC374=Wuhan1/Nyubai) for Type II resistance. Type II resistance (disease spread within the spike) was phenotyped in the greenhouse using single floret injections with a mixture of macro-conidia of three virulent strains of *Fusarium graminearum*. Due to the limited heterogeneity in the genetic background of the crosses and based on the spread of infection, fixed recombinants in the interval between molecular markers XGWM533 and XGWM493 on 3BS could be assigned to discrete 'resistant' and 'susceptible' classes. The phenotypic distribution was bimodal with progeny clearly resembling either the resistant or susceptible parent. Marker order for the two maps was identical with the exception of marker STS-3BS 142, which was not polymorphic in the HC/98 population. The major gene *Fhb1* was successfully fine mapped on chromosome 3BS in the same location in the two populations within a 1.27 cM interval (S/T) and a 6.05 cM interval (HC/98). Fine mapping of *Fhb1* in wheat provides tightly linked markers that can reduce linkage drag associated with marker-assisted selection of *Fhb1* and assist in the isolation, sequencing and functional identification of the underlying resistance gene.

Poster 34.

Qualitative mapping of the *Fusarium* head blight resistance gene on chromosome 6B in bread wheat (*Triticum aestivum* L.)

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Fusarium head blight (FHB) is one of the most important fungal wheat diseases worldwide. Understanding the genetics of FHB resistance is key for plant breeders to facilitate the introgression of different FHB resistance genes into adapted wheat. Our objective was to study the FHB resistance gene on chromosome 6B, quantify the phenotypic variation and qualitatively map the resistance gene as a Mendelian factor. FHB resistant parent, BW278 (AC Domain*2/Sumai 3), was used as the source of this gene. A large mapping population, BW278 x Foremost was developed using single seed descent, which segregated for three major FHB resistance QTLs (3BSc, 5A, 6B). Molecular markers on chromosome 6B (XWMC104, XWMC397, and XGWM219), 5A (XGWM154, XGWM304, and XWMC415), and 3BSc (XWMC78, XGWM566, and WMC527) were amplified on approximately 2,000 F₂ derived F₇ recombinant inbred lines (RILs). 95 RILs were selected that were fixed susceptible for QTLs 3BSc, 5A and recombinant for 6B. Type I (initial infection = incidence) and Type II resistance (disease spread within the spike = severity) was measured on the 95 RILs at two locations (Carman, Manitoba and Glenlea, Manitoba) over two years (2003 and 2004) using spray inoculations with a mixture of macro-conidia of virulent strains of *Fusarium graminearum*. Disease incidence and disease severity were each measured using a 1-10 scale; and a visual rating index (VRI) was calculated (VRI = Incidence x Severity) for each line. The phenotypic distribution was bimodal with lines clearly resembling either the resistant or susceptible checks. Preliminary qualitative mapping results of the *Fusarium* head blight resistance gene on chromosome 6B indicates the gene is close to marker XGWM644.

Poster 35.

Comparison of marker-assisted and conventional selection for resistance to *Fusarium* head blight in a backcross breeding program in wheat.

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Marker-assisted selection (MAS) is an efficient tool in plant breeding, especially for traits controlled by multiple genes, such as resistance to *Fusarium* head blight (FHB) of wheat. The objective of this study was to determine the efficiency of MAS relative to conventional visual selection (CVS) for resistance to FHB in spring wheat. BW 301, a line susceptible to FHB, was crossed with HC 374, a line resistant to FHB 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 genes 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. Again, the two selection methods were applied in their respective BC₂F₁ populations. Selected FHB resistant plants were advanced to the F₂ in each population, except that single seed descent (SSD) was used for the CVS population. The MAS population consisted of 26 BC₂F₂:F₃ families and the CVS population consisted of 67 BC₂F₃- SSD families. These two populations were evaluated for reaction to FHB reaction in the field in 2004 and 2005. One row plots (1 meter long) were used with three replications for the MAS population and one replication for the CVS population. 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. These results confirm the expectation that that MAS is more efficient than CVS for resistance to FHB in this backcross breeding program in wheat.

Keywords: *Fusarium* head blight, marker assisted selection, conventional visual selection, wheat

Poster 36.

Monitoring of *Fusarium* species in soybean roots using DNA arrays and reverse dot-blot hybridization.

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Sensitive and specific molecular assays facilitate direct monitoring of plant diseases caused by *Fusarium* species without the need for isolation and morphological identification of pure cultures. We developed a DNA array including 71 species or clade specific oligonucleotides based on EF1-alpha sequences obtained from GenBank, from our own research, and unpublished sequences from K. O'Donnell, for use in a reverse dot blot assay using labelled PCR products amplified directly from soybean roots. The selection of species was based on a survey of 116 commercial soybean fields in eastern Ontario and Québec, Canada in 2001 and 2002. A set of 219 *Fusarium* isolates was selected from a total of 876 field strains, and identified using partial sequences of the EF-1 alpha gene and morphological observations. Eleven groups were found, namely, the *F. oxysporum* complex, *F. solani* complex, *F. avenaceum* complex, *F. graminearum* complex, *F. sporotrichioides*, *F. tricinctum*, *F. equiseti* complex, *F. proliferatum*, *F. sambucinum*, *F. acuminatum* and *Fusarium* sp cf *merismoides*. The results using the DNA array assays were consistent with the results from direct isolation from the same roots. The technique allowed direct identification of the *Fusarium* species implicated in sudden death syndrome of soybean disease in southern Ontario.

Dépistage des espèces de *Fusarium* présentes dans les racines de soja à l'aide de la technique d'hybridation avec les macropuces à ADN

La sensibilité et la spécificité des analyses moléculaires facilitent le dépistage des maladies causées par les *Fusarium* spp. en éliminant les étapes d'isolement et d'identification morphologique des cultures pures. A partir des séquences du gène EF1-alpha déposées dans la base de données de GenBank, celles provenant de notre étude et des séquences non publiées de K. O'Donnell, nous avons mis au point 71 oligonucléotides spécifiques que nous avons utilisé dans un essai de détection des espèces de *Fusarium* présentes dans les racines de soja par hybridation sur macropuce à ADN. Le choix des espèces cibles était basé sur les souches isolées d'échantillons de plantules de soja provenant de 116 champs commerciaux de l'est de l'Ontario et du Québec en 2001 et 2002. Sur un total de 876 isolats obtenus, 219 ont été identifiées par séquençage d'une portion du gène EF1-alpha et par observations morphologiques. Nous avons trouvé 11 groupes ou espèces de *Fusarium*, notamment les complexes de *F. oxysporum*, *F. solani*, *F. avenaceum*, *F. graminearum*, *F. equiseti* et le *F. sporotrichioides*, le *F. tricinctum*, le *F. acuminatum*, le *F. proliferatum*, le *F. sambucinum*, de même que le *Fusarium* sp. aff. *merismoides*. Les résultats de détection des *Fusarium* par macropuce à ADN concordaient avec ceux obtenus par isolement. La technique moléculaire nous a permis de détecter l'espèce de *Fusarium* impliquée dans la mort subite du soja dans le sud de l'Ontario.

Poster 37.

A systems approach to *Fusarium graminearum* biology

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The broad host pathogen *Fusarium graminearum* infects the reproductive structures and stalks of numerous cereal crops. *Fg* infection is economically costly and the associated mycotoxin contamination of grains creates serious health issues. While extensive study has been focused on the effects of these mycotoxins, the genetic basis of *Fg* infection in economically important crops such as wheat, maize and barley has proven to be difficult and arduous. As the completed genome of *F. graminearum* is being annotated, we are looking ahead to identifying regulatory networks that control its pathogenesis. Tri- 6, Tri-10, and Tri-15 have been identified as having regulatory function within the trichothecene pathway leading to these devastating mycotoxins. We are interested in the molecular components that mitigate, and affect these and other regulators of pathogenicity. We are utilizing the Gateway recombinant system to construct a protein hierarchy map using both *in vitro* (GST pull downs, Yeast 2-hybrids, and IPs) and *in vivo* (TAP and epitope tagging of Tri-6, Tri-10, and Tri-15) strategies. The genetically tractable system of Arabidopsis will allow us to place the *Fg* infection process within a defined genetic background that, in turn, will allow us to specifically target a particular pathway in the economically important crops. The phenome map overlaid with the interactome maps will lead to better understanding of disease resistance and thus will enable us to design novel strategies to combat diseases.

SESSION III - EPIDEMIOLOGY

Aerobiology and Regional Epidemiology of *Gibberella zeae*

Gary Bergstrom, Cornell University

***Fusarium* Head Blight: The Alberta Experience, 1995-2004**

Kelly Turkington, Agriculture and Agri-Food Canada

Previous Crop Residues and *Fusarium* Head Blight on Cereals

Sylvie Rioux, CÉROM

A Trichothecene Chemotype Cline in Canada and the Changing Nature of FHB in North America

Todd Ward, United States Department of Agriculture

**Update on Studies for Biological Control of *Fusarium graminearum*/
Gibberella zeae in Canada**

Jeannie Gilbert, Agriculture and Agri-Food Canada

Aerobiology and Regional Epidemiology of *Gibberella zeae*

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Ascospores of *Gibberella zeae* rely on atmospheric motion systems for transport to the florets of susceptible cereal plants. We characterized the processes of spore liberation, horizontal transport, and deposition. We measured the distance that ascospores were discharged in still air, and determined that spore liberation into turbulent air currents is favored by spore release during daylight hours when peak discharge from perithecia on corn residues also occurs. Ascospore survival in the atmosphere is on the order of hours to days. Using remote-piloted aircraft, we documented the abundance of viable spores of *G. zeae* in the planetary boundary layer of the atmosphere, 60 m above the surface of the earth, at all times of the day and night under a broad range of meteorological conditions. Viable spores were deposited in cereal fields by gravitational settling mainly at random and predominantly at night. The temporal uncoupling of peak spore release and deposition suggests that inoculum in cereal fields may originate from distant sources. Genotypic diversity was extremely high in atmospheric populations of *G. zeae*. Our findings suggest that atmospheric populations of *G. zeae* are an abundant, well-mixed, and diverse source of inoculum for regional epidemics of *Fusarium* head blight.

***Fusarium* Head Blight: The Alberta Experience, 1995-2004**

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1. The presence of *F. graminearum* in barley and wheat grain, central and northern Alberta 1995-1997

From 1995 to 1997 a survey was conducted to determine whether *Fusarium graminearum* was routinely present in seed samples taken directly from fields just prior to harvest and to examine samples for the presence of other *Fusarium* spp. A secondary objective was to determine the presence and level of other barley and wheat pathogens that may be seed-borne. From 1995 to 1997 heads were collected from a total of 160 barley and 188 wheat fields from the Peace River region to the Three Hills area of Alberta, Canada. After threshing, 100 seeds from each field were surface-sterilized, incubated on potato dextrose agar and examined for the presence of the following pathogens; *Fusarium graminearum*, other *Fusarium* spp., *Cochliobolus sativus*, *Pyrenophora tritici-repentis*, *P. graminea*, *P. teres*, and *Stagonospora nodorum*. *Fusarium graminearum* was not detected in any seed samples. The most common *Fusarium* sp. isolated was *F. avenaceum*. Maximum seed infection levels with *F. avenaceum* in a single field were 51 and 37% for barley and wheat, respectively. Substantial levels of seed-borne *P. teres* were found in barley with maximum infection levels of 82, 81, and 89% in 1995, 1996, and 1997, respectively. *Stagonospora nodorum* was also commonly found in both barley and wheat seed with maximum infection levels of 61 and 54%, respectively. *Pyrenophora tritici-repentis*, *P. graminea* and *C. sativus* were generally present at low levels.

2. The presence of *F. graminearum* from fusarium head blight symptoms, and cereal, grass and corn residues, Alberta 2001-2003

Although in-crop head surveys and assessments of harvested grain have suggested that *F. graminearum* is a relatively uncommon fungal species to find in Alberta cereal grain there is still the potential for this pathogen to be infesting crop and grass residues. *Fusarium graminearum* has an extensive host range that includes all commonly grown small grain cereals, corn, and numerous wild and tame grass species. In addition, *F. graminearum* is capable of causing seed rot, seedling blight and crown/root rot in barley and wheat and thus could be present in infested cereal crown and root tissues. The objective of this study was to assess the presence of *F. graminearum* from a variety of sources including FHB symptoms, cereal node and crown tissues, grass nodes, and corn

nodes. Since 2001, *Fusarium* spp. associated with fusarium head blight symptoms in wheat, and cereal, grass and corn residues in Alberta have been evaluated. Fusarium head blight surveys typically covered an area from Barrhead, east to Provost, and south to Lethbridge. Fields also were surveyed in the Peace River region of Alberta. Counts of 300 heads were taken in each of 163 fields in 2001, 60 in 2002, and 128 in 2003 and the incidence of FHB determined. Crop residues were surveyed in 2001, from 50 and 3 cereal fields in central and southern Alberta, respectively, while 7 corn fields were surveyed in southern Alberta. In 2002, 20, 11, and 30 cereal fields were surveyed for crop residues in central Alberta, the Peace River region, and southern Alberta, respectively. In central Alberta, 19 corn fields were surveyed, while 12 were surveyed in southern Alberta in 2002. A total of 52, 33, and 43 cereal fields were surveyed in central Alberta, the Peace River region, and southern Alberta, respectively in 2003. In 2003, 31 and 13 corn fields were surveyed in central Alberta and southern Alberta, respectively. Cereal crop residues were also sampled by Alberta Research Council staff in a total of 85 cereal fields and 2 corn fields in east-central Alberta from 2001 to 2003.

In 2001 and 2002, over 75% of the wheat fields surveyed did not have any plants with visible symptoms of FHB. In those fields with plants exhibiting symptoms the yearly average incidence of FHB ranged from 1.4-2.0%. The maximum observed incidence per field was 10.7, and 6.3% for 2001, and 2002, respectively. Most wheat plants exhibiting symptoms of FHB in 2001 were in fields located in central Alberta. In 2002, 6 of the 8 fields with suspect plants occurred in southern Alberta. In 2001, the maximum incidence levels tended to occur in fields northwest of Edmonton, in the Barrhead, Westlock, and Morinville areas. Most of the symptoms in 2001 were due to *F. avenaceum* and some *F. culmorum*, while *F. graminearum* was isolated from one head from a field in southern Alberta. In 2002, *F. graminearum* was found associated with symptoms in three fields from southern Alberta, while symptoms in the remaining five positive fields were caused by *F. culmorum* and *F. avenaceum*. In 2003, 49.2% of the wheat fields surveyed had no plants with visible symptoms of FHB; the remainder had symptoms similar to those typical of FHB. Most crops surveyed in the Peace River region (11 of 17) and areas south of Edmonton towards Lacombe (17 of 20) had no symptoms of FHB. The mean incidence of FHB in affected crops in the Peace region and crops south of Edmonton towards Lacombe was < 0.5%, while the incidence for individual affected crops ranged from 0.3 to 0.7%. In the area immediately around Edmonton FHB symptoms occurred in 22 of 25 crops with an overall average incidence of 3.4% in affected crops; the incidence for individual affected crops ranged from 0.3 to 9.3%. In the area east of Edmonton FHB symptoms occurred in 13 of 23 fields surveyed, with an overall average incidence of 4.2%. In southern Alberta, 21 of the 43 crops surveyed had FHB symptoms with an overall average incidence of 2.5%; average incidence for individual affected fields ranged from 0.3 to 9.3%. Of the 43 crops surveyed in southern Alberta, 35 were under irrigation, while 8 were dryland crops. Only one of the 8 dryland crops had symptoms of FHB with an incidence of 0.7%. In comparison, 20 irrigated crops had symptoms of FHB at an average incidence of 2.6%, and incidences for individual affected fields ranged from 0.3 to 9.3%. In 2003, all visible symptoms of FHB in central Alberta (including fields east of Edmonton) and the Peace River region were due largely to *F. culmorum*, *F. poae*, and *F. avenaceum*, with the number of heads per field (out of 300) affected by these pathogens ranging from 1 to 14 heads. *Fusarium graminearum* was not recovered from these samples. In southern Alberta, the head symptoms in 10 of the 21 affected fields were due solely to *F. graminearum*, with 1 to 24 heads involved.

All these fields were under irrigation. Symptoms in other FHB-positive fields were ascribed to *F. culmorum* and *F. avenaceum*, with the number of heads per field affected by these pathogens ranging from 1 to 17.

From 2001 to 2003, *F. graminearum* was typically not detected in cereal crown and node tissues in most fields surveyed in central Alberta and the Peace River region. However, in 2003 a single piece of cereal node tissue from one field in the Peace River region was found to have a culture typical of *F. graminearum*, but a viable culture was not available to confirm whether it was *F. graminearum* or *F. pseudograminearum*. *Fusarium graminearum* was detected from crown tissues in 3 fields in 2002 and 4 fields in 2003 from southern Alberta. Levels of detection of *F. graminearum* in these crown tissues ranged up to 50%, with most fields being less than 10%. In southern Alberta, *Fusarium graminearum* was detected from node tissues in 2 fields each in 2001, 2002, and 2003. Levels of detection of *F. graminearum* in these node tissues ranged up to 46.7%, with most fields being less than 10%. *Fusarium pseudograminearum* tended to be more commonly found compared to *F. graminearum* and was detected mainly at low levels in central and southern Alberta. In addition, *F. pseudograminearum* was more frequently found associated with crown tissues compared to the node tissues that were tested. Overall, *F. avenaceum* and *F. culmorum* were among the most common *Fusarium* spp. associated with cereal crown and node tissue in all regions of Alberta. The frequency of detection of both these species tended to be slightly higher from crown tissues versus node tissues. Maximum levels of crown or node infection with *F. avenaceum* and *F. culmorum* ranged up to 100% and 96%, respectively. For grass nodes, *F. graminearum* was only recovered from a total of 3 sites located in southern Alberta in 2001 and 2003, with maximum levels of infection within these sites being less than or equal to 4%. *Fusarium pseudograminearum* was also found at low levels at some sites in southern Alberta in all three years and at a single site in the Peace River region in 2003, but was not detected on grass nodes from central Alberta. Overall, *F. avenaceum* tended to be the most common *Fusarium* spp. detected from grass node tissues, with maximum levels of infection that ranged up to 52% in some regions. *Fusarium culmorum* was less commonly found than *F. avenaceum*. In general, the level of grass node infection with *Fusarium* spp. tended to be lower compared with cereal and corn tissues. *Fusarium graminearum* more commonly found associated with corn node tissues compared to cereal and grass residues in southern Alberta. Of the seven fields surveyed in 2001, 3 fields were found to have detectable levels of *F. graminearum*, with average infection levels of 10 to 40%. In 2002, 8 of 12 corn fields surveyed in southern Alberta had detectable levels of *F. graminearum* in the lower node tissue that was tested. Node infection levels in these fields ranged from 10 to over 40%. In 2003, all 13 corn fields surveyed in southern Alberta had detectable levels of *F. graminearum* in the node tissue that was tested. The average level of infection was 28% with a range of 4.0 to 60.0%. In contrast, *F. graminearum* was not isolated from any of the node tissues that were sampled from corn fields in central Alberta in 2002 and 2003. Of the other *Fusarium* spp., *F. culmorum* tended to be the most commonly found *Fusarium* spp. in corn node tissues from southern Alberta, while *F. avenaceum* tended to be most prevalent in central Alberta. Other *Fusarium* spp. that were recovered from cereal, grass and corn residue at generally lower levels included *F. sporotrichioides*, *F. acuminatum*, *F. poae*, and *F. equiseti*. Surveys by Alberta Research Council staff indicated that *F. graminearum* was rare in east-central Alberta cereal fields from 2001-2003. Over all three years, *F. avenaceum* was most frequently isolated, followed by *F. culmorum* and *F. equiseti*. A number of

Fusarium species were only isolated a few times, and should thus be considered rare. This includes *F. graminearum*, only three colonies of which were found from wheat residues; one from a field in Beaver County near Holden, and two colonies from the same field north of Vegreville in 2002. No *F. graminearum* was found in 2003.

3. Characteristics of Alberta wheat fields found to have detectable *F. graminearum* as part of the Canadian Grain Commission, harvest survey, 2002 and 2003

Over the past several years the Canadian Grain Commission (CGC) has observed an increasing frequency of *Fusarium graminearum* (Schwabe) (*Fg*) associated with fusarium damaged kernels collected from Alberta wheat samples submitted as part of their yearly harvest survey. Characteristics of these *Fg*-positive (+ve) fields were collected anonymously from Alberta producers to develop a better understanding of factors contributing to the appearance and development of *Fg* in Alberta. In 2002 and 2003, 40 and 45 Alberta producers with *Fg*+ve grain samples were contacted, respectively, and fields were classified according to wheat class, irrigation, tillage regime, previous crop, and frequency of host crops in the previous 4 years. In 2002, 17 of the producers contacted responded to the survey questions that were sent out, while in 2003, 19 of the producers responded. Over 90% of the *Fg*+ve fields were planted to wheat cultivars with either a poor or very poor rating for fusarium head blight resistance.

In 2002 and 2003, 41% and 70%, respectively of the *Fg*+ve fields were amber durum. Considering only the irrigated region of Alberta, irrigation was reported for 8 of 11 and 16 of 17 fields in 2002 and 2003, respectively. Over 80% of the fields with *Fg*+ve samples were under either conventional or minimum tillage. A slightly higher percentage of fields had a non-host versus host (56.2 vs. 43.8%) as the previous crop in 2002, while in 2003 a similar trend was observed. In 2002 and 2003, most of the *Fg*+ve fields (> 80%) had 2 or 3 host crops planted during the previous 4 years. In general, susceptible wheat classes/cultivars, irrigation and rotations with less than 2 years between host crops were common practices for producers with *Fg*+ve grain samples.

4. Forecasting the potential distribution and relative abundance of *fusarium* head blight using CLIMEXJ modelling under current long-term climatic conditions, for an irrigated scenario, and with above-average moisture conditions

Fusarium graminearum (*F.g.*), the main causal agent of fusarium head blight, is well-established in the eastern prairies, but its appearance in grain samples from central and western regions has raised concerns about the broader risk to cereal production in the central and western prairies. The computer simulation program, CLIMEX™, was used to develop a model to predict potential distribution and severity of *F.g.* in western Canada under current long-term climatic conditions as well as under an above-average precipitation scenario. The model was also extended to examine the effect of irrigation. Based on current conditions, the model predicted that *F.g.* could expand to encompass large areas of Saskatchewan and Alberta. In particular, the Edmonton region could be expected to have disease levels that approach those currently experienced in the eastern prairies. The model demonstrated that *F.g.* was more sensitive to soil moisture than temperature. Irrigation may compensate for dryer atmospheric conditions in some regions resulting in disease levels similar to those occurring in the eastern prairies. This is consistent with observations from FHB symptom surveys and irrigation studies recently conducted in southern Alberta. There was an expansion of areas where FHB may occur at economic levels with above-average (120% of normal) rainfall during the growing season. This is consistent with observations in southeastern Saskatchewan where FHB

caused by *F. graminearum* increased in prevalence from the mid to late 1990=s into 2000 and 2001, due to more favourable moisture conditions during some years.

5. Spilled grain and potential association with the presence of *fusarium* head blight and *Fusarium graminearum* in adjacent grassy areas and cereal fields, Alberta 2002-2003

Since the late 1990's and early 2000's concerns have been expressed regarding the movement of *F. graminearum* infected feed grain into Alberta, especially in terms of spillage that has occurred at unloading sites and along roadways. Spillage of feed grain, grain screenings or feed pellets that contain viable *F. graminearum* could potentially introduce this pathogen into areas where it is not common. The fungus readily produces fruiting structures on infected cereal grain and these structures forcibly release ascospores into the air where they may be carried by wind currents to introduce this pathogen into adjacent cereal or corn fields, or grassy areas. In 2002 and 2003, grain spillage sites were surveyed in southern Alberta. A total of 5 sites were surveyed in July of 2002 and 6 in May of 2003. The sites in 2002 were spillage sites along railway tracks, while in 2003, 5 of the 6 sites were along railway tracks, while the last site was a small roadside spillage site of feed barley. A seventh site (also sampled in 2002) was also sampled, but only for grass residues in 2003. Adjacent grassy areas and cereal fields were also surveyed for the presence of *F. graminearum* in residues and from fusarium head blight symptoms present on wheat heads. The total average amount of grain m⁻² ranged from 65 to 1702 g m⁻² in 2002 and 2003. The most common types of spilled grain were barley and corn. Low levels of *F. graminearum* were found associated with the spilled corn with average % incidence ranging from 0 to 1.4% in both years. Isolation data were combined for the small grain cereals. The incidence of cereal grain infection with *F. graminearum* was higher in 2002 and ranged from 1.6 to 14.5%, while in 2003 it ranged from 0 to 2.1%. No consistent trends were observed for FHB incidence with distance from the spillage sites. *Fusarium graminearum* was found in cereal crown and/or node tissues taken from residues in fields adjacent to spillage sites 1 and 2, and *F. pseudograminearum* from fields adjacent to spillage sites 3 and 5 in 2002. No *F. graminearum* was detected in crown or node tissues taken from the cereal fields adjacent to spillage sites in 2003. In both 2002, and 2003, no *F. graminearum* was detected in grass node tissues collected from areas immediately adjacent to the spillages sites. Results from 2002 and 2003 did not demonstrate that *F. graminearum* was consistently associated with grass or cereal residues, or FHB symptoms collected from areas or fields adjacent to spillage sites in southern Alberta, including sites where *F. graminearum* had been detected in spilled grain. Generally, dry weather conditions and low levels of *F. graminearum* infection of spilled grain at some sites, may have precluded the sporulation, dispersal, establishment, and appearance of this pathogen in adjacent grassy areas and cereal fields. Although *F. graminearum* was not consistently found in adjacent grassy areas and cereal fields in southern Alberta, spillage of infected grain, especially with high levels of *F. graminearum*, may be more of a concern in moister regions of Alberta. In these regions perithecia and ascospore production may occur more frequently and the potential for infection of adjacent grass or cereal tissues could be higher due to more favourable moisture conditions. Spilled grain or infected feed could also be a potential concern if they were directly spread onto fields where *F. graminearum* could attack and infect seedlings, root systems and other tissues of cereals or corn that may be planted in these fields or potentially colonize senescing plant tissues.

Previous Crop Residues and *Fusarium* Head Blight on Cereals

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In eastern Canada fusarium head blight (FHB) has been prevalent on wheat since the 1980's. In barley it has been a problem since the late 90's and more recently it has also affected oat production. Researchers have explained the intensification and expansion of the disease by: i) shorter crop rotations; ii) the use of reduced tillage which leaves more crop residues on the soil surface than traditional ploughing; iii) the cultivation of highly susceptible cereal cultivars, including corn; and iv) warmer weather.

It is recognized that *Fusarium* spp. overwinter on corn and wheat residues and produce spores that can infect inflorescences of the cereal cultivated the following year. Some studies have demonstrated that *Fusarium* spp. can produce spores on residue left on the soil surface, but not on buried residues (Khonga and Sutton 1988; Inch and Gilbert 2003) even though *Fusarium* spp. can survive there as mycelium.

Fusarium head blight may be affected by the amount, nature, and rate of decomposition of residues left at the soil surface, which in turn are influenced by tillage system, previous crop and weather. We have known for a long time that crop rotation is effective in maintaining or increasing yield by decreasing soilborne pathogens. Many of the studies reported in Dill-Macky and Jones (2000) and Champeil *et al.* (2004) show that the impact of FHB in wheat may be decreased by growing non-host species, such as soybean, sugar beet, flax, and alfalfa in the preceding year. Schaafsma *et al.* (2001) also reported that the DON content in wheat was higher when the previous crop was corn than when it was soybean or wheat.

Reduced tillage might also logically be thought to increase FHB incidence compared with ploughing because more residue is left on the soil surface. However, even though some studies show that, indeed, reduced tillage systems can increase FHB in wheat (McMullen *et al.* 1997; Rossi *et al.* 2004; Teich and Hamilton 1985; Yi *et al.* 2001), others show the lowest FHB index is under no-till (Fernandez *et al.* 2005) or that tillage systems have no effect (Miller *et al.* 1998; Schaafsma *et al.* 2001; Teich and Nelson 1984).

Some studies have demonstrated interactions between tillage system and previous crop in their effect on FHB content in wheat (Del Ponte *et al.* 2003; Dill-Macky and Jones 2000; Schaafsma *et al.* 2005). After a corn crop, DON contamination in wheat was higher with chiseling and no-till than with ploughing, but after a soybean or wheat crop tillage had no effect (Dill-Macky and Jones 2000). Recently it has been reported that in regions where reduced tillage is widely practiced and where corn is prevalent, the background of viable airborne spores may provide enough inoculum to cause infection (Del Ponte *et al.* 2003; Schaafsma *et al.* 2005; Schmale *et al.* 2005); therefore crop rotation might have little effect on FHB. However, even in regions such

as southwestern Ontario (Schaafsma *et al.* 2005) and New York State (Del Ponte *et al.* 2003) FHB in wheat was shown to be more severe after a previous crop of corn with reduced tillage than after a previous non-host crop with conventional tillage. In Ontario, this result applied only to large fields (8-16 ha) and there was no effect of adjacent crops (non-host, corn or wheat).

We conducted an experiment at Saint-Hyacinthe in a major corn-growing area of southwestern Québec, where reduced tillage practices are increasing. Even though the plots were small (3 m x 18 m) and close to each other (30 cm apart), we observed effects of previous crop and tillage. For example, in 2003, a year with high disease pressure, DON content in wheat was higher after corn than after soybean, and was higher with minimum tillage than with conventional tillage (Table 1). It should be noted, however, that glyphosate was applied in the spring of 2002 to the plots under minimum tillage. Since Fernandez *et al.* (2005) recently reported that glyphosate application in the previous 18 months can be associated with higher levels of FHB and fusarium damaged kernels in wheat, it is possible that glyphosate application contributed to the high DON levels observed under minimum tillage in our study. Based on these results, avoiding sowing wheat after corn should be recommended to growers, especially with reduced tillage, which is generally in conjunction with glyphosate applications. The economic benefit will be greatest in years highly conducive to the disease.

Table 1. Effects of rotation and tillage on DON content (ppm) in wheat at Saint-Hyacinthe in 2003

Rotation ¹	Tillage ²		Rotation
	Minimum	Conventional	mean
S/W/C/S/W	3.3	2.5	2.9 y
C/W/S/C/W	4.0	3.5	3.7 z
Tillage mean	3.6 a	3.0 b	

¹ C = corn, S = soybean, W = wheat; the first crop in the sequence was seeded in 1999.

² Conventional = moldboard ploughing in fall and two cultivator operations in spring; minimum = no-till in fall and one disk harrowing in spring followed by two cultivator operations.

Mean values followed by the same letter in the column or in the row are not significantly different ($P < 0.05$).

The above recommendation will be useful to wheat growers in areas where corn is well-adapted and prevalent in rotations. However, in other areas of Quebec, most rotations include barley. At Normandin, located in an important area of barley production 300 km north of Québec city, experiments were conducted to measure the effect of crop rotation and tillage practices on FHB in barley. The results of three experiments are summarized below.

Several management systems were applied to barley, oat and wheat fields in the fall of 2002 and the spring of 2003, and the DON content of grain harvested in 2003 was analyzed. The same experiment was conducted in 2003 and 2004 in other fields. The management systems consisted of 14 combinations of straw removal vs. no removal; no tillage vs. chiseling vs. and moldboard ploughing in the fall; disk harrowing vs. cultivation in the spring, and direct seeding after no fall tillage. The trials were not inoculated with *Fusarium* and glyphosate was applied in every

treatment the year preceding DON assessment. The results showed that the growing season was more favorable in 2003 than in 2004 to DON accumulation in grain, and that barley was more contaminated than oat or wheat in the region. The major effect of weather during the growing season on FHB development is recognized worldwide. However, differences in the relative susceptibility of cereal species to FHB appear to vary between countries or regions (Edwards, 2004). According to Edwards (2004) this may be due to differences in the genetic pool among breeding programs, and in environmental and agronomic conditions under which crops grow.

The data also showed effects of cultural practices on FHB in two trials: barley in 2003 and wheat in 2004. In these trials, DON content was lower with fall ploughing than with the reduced tillage systems tested (chiseling and no tillage). Also and surprisingly, straw removal increased DON content in no tillage treatments. This last result might be explained by the finding of Khonga and Sutton (1988) that stem residues are less favorable to ascospore production than spikelets and grains. Thus, in the no straw removal + no tillage treatments dissemination of spores produced on spikelets and grains at the soil surface could have been impeded by the straw.

In another 2-year experiment, DON content in barley was studied in relation to a previous crop consisting of a barley monoculture vs. a previous mixed forage crop [orchard grass (*Dactylis glomerata* L.) and red clover (*Trifolium pratense* L.)]. Two tillage systems (moldboard ploughing vs. chiseling) and two fertilizers (mineral vs. liquid dairy cattle manure) were also compared, using a split-split plot design. The previous crop was in the main plots, with tillage system in the subplots and fertilizer in the sub-subplots. The forage crop was harvested the previous year when the orchard grass was at the beginning of heading, and again 5 weeks later. Glyphosate was applied in every treatment the year preceding DON assessment. In 2003, DON content was significantly higher for the forage in chiseling than for the barley in ploughing. Fertilization had no significant effect on DON content. In 2004, there was an effect of each treatment on the DON content of the barley grain, but no interactions among treatments. A previous forage crop, chiseling, and mineral fertilization were more favorable to DON accumulation than a previous barley crop, ploughing, and liquid manure, respectively. Note that the results of both 2003 and 2004 indicated that ploughing crop residues may help to decrease FHB. They also indicated that forage crop residues may be more conducive to FHB than barley residue. This could have major significance for dairy and beef producers who usually plant barley after a 3-4-year forage crop. Data are needed on additional grasses as the previous crop, particularly common timothy (*Phleum pratense* L.), which is the most prevalent grass in forage crops in Québec.

In 1999, a trial was initiated for comparing the effect of 4-year crop rotations and two tillage systems on crop productivity and soil quality and since 2001 DON content has been analyzed in grain from this trial. Crops in the rotations were barley (B), canola (C), and pea (P) and the seven rotations were BBBB, CPBB, PCBB, BCPB, BPCB, CBPB, and PBCB. There were two tillage treatments, conventional (moldboard plough) and reduced tillage (chisel) in the fall, giving a total of 14 sequence + tillage treatments. The experiment had a 4-replicate split-plot design with tillage practices in the main plots and crop rotations in the subplots. The trial was subject to natural infection and glyphosate was applied every year in each treatment.

In 2002 and 2004, the treatments had no effect on the DON content of harvested barley, which

was lower than 1.0 ppm. In 2001 and 2003 significant rotation x tillage interactions were observed. From the 2003 results we can extract several general points. DON content was significantly higher with chiseling than with ploughing, but only in rotations where barley was the previous crop, i.e. BBBB + chisel, PCBB + chisel and CPBB + chisel. These three treatment combinations (12.7-15.7 ppm) were significantly different from 10 of the other 11 combinations (4.6-6.4 ppm). The exception was CBPB + chisel (8.2 ppm) which was not different from BBBB + chisel. These results indicate that when barley is the previous crop, ploughing the residues may help to decrease DON content in barley compared to chiseling. The results also indicated that ploughing was not beneficial when the previous crop was canola or pea. As noted previously, however, the effect of a pea crop inserted between 2 years of barley (CBPB) was not clear, especially with chiseling. Other studies conducted in western Canada (Gilbert *et al.* 2004; Fernandez *et al.* 2004) have shown that pea residue can be a favorable substrate for *Fusarium* spp. including *F. graminearum*. Canola was also reported to be a favorable substrate by Fernandez *et al.* (2004), whereas in the Normandin trial, even 1 year of canola inserted between two barley crops in the rotation reduced FHB. This demonstrates that regional experiments are needed to establish recommendations to producers for agronomic practices.

Conclusions

Corn, small-grain cereal, and even grass residues left at the soil surface appear to be a ‘good’ substrate the following year for *Fusarium* spore production. Planting cereals on such residues increases the risk of getting a DON content in grains higher than market requirements. Field crop producers in Québec who use reduced tillage should not plant cereals the year following corn or any other cereals and be cautious about pea as the previous crop. Two years of non-host crops before a cereal should be seriously considered with reduced tillage. More data are needed about forages as previous crops, especially on common timothy, the most widely used grass in the province.

In experiments in 2003, a year very favorable to FHB development in Québec, even the lowest DON content exceeded the level of 2.0 ppm set for wheat by the food industry or the level of 1.0 ppm required for barley by the swine industry. This emphasizes that producers should use all additional means they can to reduce risk, namely planting less susceptible cultivars, seeding early, preventing lodging, timely harvesting, maintaining the moisture content in grains at 13 % or less after harvest, and, finally for wheat, using a fungicide at flowering when the risk of infection justifies it.

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A Trichothecene Chemotype Cline in Canada and the Changing Nature of FHB in North America

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Our previous phylogenetic and molecular evolutionary analyses demonstrated that the primary etiological agent of FHB, the morphospecies *F. graminearum*, actually comprises at least 11 distinct species. In addition, we have demonstrated that the three trichothecene chemotypes segregating within these species have been maintained by balancing selection and are adaptive. Until recently, it appeared that *F. graminearum* (*sensu stricto*) isolates with a 15ADON chemotype were the only significant cause of FHB in North America. However, molecular surveillance with a multilocus genotyping assay for FHB species and chemotype determination has revealed an East-West chemotype cline in Canada and evidence that *F. graminearum* isolates with a 3ADON chemotype may be displacing those with a 15ADON chemotype. In addition, 3ADON isolates have been found to produce significantly ($P < 0.001$) higher levels of trichothecene than those with a 15ADON chemotype. Similar patterns have been observed in the Northern Plains and within the Northeast U.S. In addition, significant populations of *F. asiaticum* and NIV-producing *F. graminearum* have been identified in the Southern U.S. Taken together, these results indicate significant changes in B-FHB pathogen composition are taking place in North America, which could have significant implications for disease control efforts and regulatory policy regarding trichothecene-contaminated grain.

Update on Studies for Biological Control of *Fusarium graminearum*/*Gibberella zeae* in Canada

Jeannie Gilbert, *Agriculture and Agri-Food Canada*

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Potential fungal and bacterial biological control agents are being assessed *in vitro* and under field conditions for their ability to control *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch), the principal cause of fusarium head blight (FHB) in North America.

The biological control potential of *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph, *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur] to suppress infection by *F. graminearum* was examined in barley and wheat. Suspensions of *F. graminearum* (5×10^4 macroconidia per ml) and *B. sorokiniana* (5×10^3 conidia per ml) were sprayed on spikes of three spring barley cultivars, AC Metcalfe (2-row malt, moderately resistant (MR) to FHB), Manley, (2-row malt, MS), and Stander (6-row malt, S) and to susceptible spring wheat cultivar, Roblin. Significant reduction of both FHB severity and DON levels were recorded on spikes treated first with *B. sorokiniana* and followed by *F. graminearum* 2-3 days later. Compared to *F. graminearum* inoculation alone, the pre-treatment with *B. sorokiniana* reduced FHB severity from a mean of 84% to 1.4% in AC Metcalfe and Manley 1.4%, and from 41% to 7.2% in Roblin (Table 1). At the same time, the mean DON for AC Metcalfe and Manley decreased from 211 ppm to 3.5 ppm, and from 19 ppm to 2 ppm for Roblin. The same trend was observed in the susceptible barley cultivar Stander, but the reductions were less dramatic. A compound from *B. sorokiniana*, which is toxic to *F. graminearum*, has been partially purified. Nuclear magnetic resonance imaging indicates that the toxin is a small organic compound that lacks any aromatic functional group and is probably a mixture of 2-3 closely related compounds.

Other organisms investigated in biological control of FHB include bacterial antagonists. *Bacillus amyloliquefaciens* strain BS6 and *B. subtilis* strain H-08-02, *Pseudomonas chlororaphis* strain PA23, and its transposon-mutant strains PA23-63 (mutation on *phzE*), PA23-314 (mutation on *GacS*), were evaluated *in vitro* for their antagonistic action against *F. graminearum*. All antagonists except strain PA23-314 inhibited pathogen growth. Although phenazine production is inhibited in strain PA23-63 it produces a second antibiotic pyrrolnitrin. Strain PA23-314 has lost most of its antifungal activity as the mutation is on the global regulator *GacS*. Isolate H-08-02 inhibited mycelial growth of *F. graminearum* by 51%. Isolates PA23, BS6 and PA23-63 inhibited the mycelial growth of *F. graminearum* up to 47, 44 and 36% respectively. Culture filtrate of antagonists H-08-02, PA23, BS6 and PA23-63 reduced the germination of macroconidia of *F. graminearum*. Antifungal compounds produced by the antagonists might be responsible for the suppression of the pathogen *in vitro* as the non-antibiotic producing strain PA23-314 was not effective in inhibiting *F. graminearum*.

Table 1. *Fusarium* head blight and deoxynivalenol (DON) in barley and wheat inoculated with *Fusarium graminearum* and *Bipolaris sorokiniana*.

FHB Severity^a

Cultivar - Barley	F. g. ^b	<u>Inoculation Treatment</u>		
		<i>F. g.</i> + <i>B. s.</i>	<i>F. g.</i> / <i>B. s.</i>	<i>B. s.</i> + <i>F. g.</i>
AC Metcalfe (2 r) MR	70.9. ^c a	68.1 a	65.5 a	1.4 b
Manley (2 r) MS	95.8 a	75.4 ab	51.5 b	1.4 c
Stander (6 r) S	70.8 a	68.6 a	82.1 a	15.1 b
- Wheat				
Roblin S	40.5	43.6	32.0	7.2

DON ppm

Cultivar - Barley	F. g.	<u>Inoculation Treatment</u>		
		<i>F. g.</i> + <i>B. s.</i>	<i>F. g.</i> / <i>B. s.</i>	<i>B. s.</i> + <i>F. g.</i>
AC Metcalfe (2 r) MR	220 a	227 a	168 a	4 b
Manley (2 r) MS	201 ab	291 a	79 b	3 c
Stander (6 r) S	140 a	155 a	142 a	20 b
- Wheat				
Roblin S	19.0	19.9	16.9	2.0

^a Percent of spikelets with typical FHB symptoms

^b *F. g.* = *Fusarium graminearum*. *B. s.* = *Bipolaris sorokiniana*. '+' = followed 72 h by, '/' = joint inoculation

^c Values in rows followed by the same letter are not significantly different at $P \leq 0.05$

However, organic volatile compounds produced by *P. chlororaphis* strain PA23 increased the mycelial growth of *F. graminearum* in contrast to the effective inhibition of *Sclerotinia sclerotiorum* mycelial growth, ascospore and sclerotia germination with organic volatile compounds 2-ethyl 1-hexanol, benzaldehyde, nonanal produced by the same bacterium. It is hypothesized that other organic volatiles produced by PA23 may be responsible for the growth enhancement of *F. graminearum*. Evaluation of the efficacy of these isolates to manage FHB under controlled conditions is in progress. The biocontrol potential of fungal antagonists including *Trichoderma harzianum* (Rifai), *Penicillium* sp. and *Aspergillus clavatus* isolates were evaluated by confrontation plate assays. Eleven *Trichoderma harzianum* isolates were paired with *F. graminearum* in Petri plates containing PDA. All but one isolate showed some ability to overgrow *F. graminearum*. Isolates T83, T51, T30, and T183 overgrew *F. graminearum* by 20 mm or more. To determine the effect of *T. harzianum* on the production of perithecia and

ascospores of *G. zea* on wheat residue, spore suspensions, or cell-free filtrates of *T. harzianum* isolates, were applied to wheat residues either 24 h before, co-inoculated, or 24 h after, inoculation with *G. zea*. Plates containing the treated residues were placed under UV light in a randomized complete block design with 4 replicates per treatment. On residues that were inoculated with either spore suspensions or cell-free filtrates of *T. harzianum*, 24 h before *G. zea*, perithecia and ascospore development were substantially reduced. Residues that were co-inoculated showed moderate reduction. No control was achieved when the residues were inoculated first with *G. zea*. Under field conditions, perithecial development on *F. graminearum*-infested straw residues was reduced by all fungal antagonists, but not to the same degree (Table 2). The standard T22 and T72 were the most effective. The effect of spore concentration and mechanisms of control are currently being investigated.

Table 2. Average number of perithecia produced on *Gibberella zea*-infested wheat stems in July and August under field conditions in 2003 and 2004.

Average number of perithecia per stem*

Treatment	2003		2004	
	July	August	July	August
T22	6	10	5	4
T72	8	9	4	4
F35	10	15	12	16
T51	12	12	6	7
F24	20	24	17	19
T88	22	15	9	8
F23	26	22	15	10
T30	35	24	14	16
F31	61	58	41	37
F7	72	85	35	44
<i>G. zea</i>	135	123	40	52

*n=24

Trichoderma harzianum: T51, T88, T72, T22

Mortierella sp.: F24, F35

Penicillium sp.: F7

Posters

SESSION III - EPIDEMIOLOGY

38.

Evidence of seed transmission of FHB caused by *Fusarium graminearum* in wheat and barley

Tomimura, K.; Nakajima, T.; Yoshida, M.

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Prediction of deoxynivalenol accumulation in barley: A regional case study
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Vigier, B.J.; Bourgeois, G.

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FusaPROG B A tool for the prediction of *Fusarium* head blight and deoxynivalenol in winter wheat

Forrer, H.-R.; Musa, T.; Hecker, A.; Vogelgsang, S.

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Different aggressiveness of isolates of *Fusarium graminearum* and *F. pseudograminearum* in causing root rot of soybean

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Detection of *Fusarium graminearum* in cereal crowns with the aid of selective media

Mise en évidence du *Fusarium graminearum* dans les collets de céréales par isolement sur milieux de culture sélectifs

Pouleur, S.; Couture, L.; Clear, R.M.

Poster 38.

Evidence of seed transmission of FHB caused by *Fusarium graminearum* in wheat and barley

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FHB is a part of complex disease, since many of the pathogens can also cause seedling blight and foot rot. However, the epidemiological relationship between the diseases is not elucidated. The ascospores produced on the debris are considered to be the major source, but role of seed born inoculum is not clear. In the western part of Japan, most of all causal species is *F. asiaticum* (= *F. graminearum* lineage 6) and its trichothecene chemotypes are 3ADON or NIV. 15ADON producer of *F. graminearum* (=lineage 7) is rarely found in the area. The seeds of wheat and barley artificially infested with the conidiospore of 15ADON producer were sown and the isolate was traced during growing season using multiplex PCR-based trichothecene chemotyping. The isolation frequency of 15ADON producer were 100 % from root and crown at the beginning of stem elongation, 7.7 % from air spore sampler at flowering and 2.8 % from diseased symptom of FHB. The difference between wheat and barley was not found. Additionally, the haplotypes of 10 VNTR markers which can distinguish individual clone of *F. graminearum* and *F. asiaticum* were corresponded completely among all isolates of 15ADON producer. These results strongly show evidence of seed transmission.

Poster 39.

Prediction of deoxynivalenol accumulation in barley: A regional case study

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A study on barley contamination with deoxynivalenol (DON) was undertaken using barley samples collected in the National mycotoxin databases by the Feeds Section of the Canadian Food Inspection Agency for Quebec during the 1992-2003 period. Mycotoxin data were linked with weather variables from neighbouring weather stations and were analyzed to detect possible associations using the Stepwise Regression Procedure (SAS[®]). Significant regression models were obtained for the Montréal, Trois-Rivières and Rivière du Loup regions. Log-transformed DON values from these regions could be estimated by regression models using weather data ($R^2 = 0.60$, $p < 0.0001$, $N = 51$). The predictive models were validated successfully using daily weather data compiled in 10-day interval. Models used mean air temperature in June, July and August, number of rainy days in June and August, and also rainfall in July and August. A regression model based on eight weather variables was validated for the Montreal region. These results confirm the importance of air temperature and rainfall in June for the onset of FHB infection in barley and its development in July and August leading to DON accumulation.

Prédiction de l'accumulation de désoxynivaléol chez l'orge: une étude régionale.

Une étude a été réalisée à partir des échantillons d'orge contaminée au désoxynivaléol (DON) archivés dans la base de données nationale sur les mycotoxines de la Section des aliments du bétail de l'Agence canadienne d'inspection des aliments pour le Québec de 1992 à 2003. Ces données de mycotoxines ont été reliées à celles de stations météorologiques avoisinantes afin de déceler les associations possibles grâce à des analyses de régression multiple progressive (SAS[®]). Plusieurs modèles de régression ont pu être établis pour les régions de Montréal, Trois-Rivières et Rivière du Loup. Les transformations logarithmiques des valeurs de DON ont pu être estimées de manière significative ($R^2 = 0,60$, $p < 0,0001$, $N = 51$) par ces modèles de régression en utilisant des données météorologiques. Les modèles de prédiction ont pu être validés à partir de données météorologiques quotidiennes compilées sur une base de 10 jours. Les modèles se sont servis de la température moyenne de l'air en juin, juillet et août, du nombre de jours de pluie en juin et août ainsi que de la pluviométrie en juillet et août. Un modèle de régression de huit variables météorologiques a été validé pour la région de Montréal. Ces résultats confirment l'importance de la température de l'air et de la pluviométrie en juin pour le démarrage de l'infection de fusariose de l'épi chez l'orge et de son développement en juillet et août pour l'accumulation de DON.

Poster 40.

FusaPROG - a tool for the prediction of *Fusarium* head blight and deoxynivalenol in winter wheat

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From 2001 to 2003, wheat samples from Swiss farms were analysed for occurrence of *Fusarium* head blight (FHB) fungi and deoxynivalenol (DON) contamination. The samples originated from ploughed or no-till fields with corn or other preceding crops and wheat varieties with a low or medium susceptibility to *Fusarium graminearum* (FG). The results were used to quantify the effects of tillage, rotation, and variety on FHB and DON and to develop a forecast model for DON contamination. DON results of the four combinations pre-crop corn or no corn and plough or no-till are used as a primary input in our model. To forecast the level of DON contamination, the input data are adjusted with an extended set of factors affecting the development of FHB such as the weather, the growth stage of wheat, the preceding crop, the soil and straw management, as well as the wheat variety. The DON model is the core of our internet information system FusaPROG. It can be used as a tool to develop cropping systems with a low risk of DON contamination, an approach especially needed for soil conservation tillage. Together with the recognition of FG infection periods, FusaPROG is also a tool for threshold-based and optimally timed fungicide applications.

Poster 41.

Different aggressiveness of isolates of *Fusarium graminearum* and *F. pseudograminearum* in causing root rot of soybean

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Fusarium graminearum Schwabe (sexual state *Gibberella zeae* (Schwein.) Petch) and *F. pseudograminearum* O'Donnell & Aoki (sexual state *Gibberella coronicola* Aoki & O'Donnell) formerly known as *F. graminearum* group 2, are important plant pathogens that are morphologically very difficult to distinguish. *Fusarium graminearum* is a causal agent of fusarium head blight (FHB) of small grain cereals and gibberella ear rot of corn, and has recently been reported to attack soybean, causing root rot and pod blight, whereas *F. pseudograminearum* is primarily a pathogen of the roots and crown of cereals. Pathogenicity tests were conducted under controlled environment conditions to determine variation in aggressiveness among isolates within and between *F.*

graminearum and *F. pseudograminearum*. In each experiment, 20 seedlings of each of three soybean cultivars were inoculated with six isolates each of the two *Fusarium* species, by placing a 2 mm diameter agar plug from 7 day-old fungal culture on the primary root at the VE stage Symptoms were rated as disease severity (DS) on a 0 to 4 scale and as percentage of reduction in shoot length (SL) and plant dry weight (DW) 10 days after inoculation. Roots and shoots of uninoculated agar plug-treated controls remained healthy throughout the experiment whereas those inoculated with the pathogen developed root rot symptoms. Within 2 days, the *F. graminearum* infected the roots and hypocotyl, forming a water soaked lesion and pink discoloration at the point of inoculation. A rapid and massive infection of the entire root was observed on cultivar Nattosan in 5 days. From 6-21 days after inoculation, severely infected tissues degraded and plants died. Disease development in cultivars Maple Arrow and AC Glengarry was similar to Nattosan, except for a slower and less extensive root rot development. All isolates caused visible infection 10 days after inoculation, but those of *F. graminearum* were significantly more aggressive than *F. pseudograminearum*. The two species caused an average DS of 2.3 and 1.1, and reduced SL by 29 and 7% and DW by 13 and 8%, respectively. Significant differences ($P < 0.05$) were found among cultivars for all parameters, among isolates of *F. graminearum* in DS and SL, and among *F. pseudograminearum* isolates in DS only, but there were no significant cultivar x isolate interactions. Further research is needed to verify genotypic difference and possible resistance in soybean to *F. graminearum*.

Poster 42.

Detection of *Fusarium graminearum* in cereal crowns with the aid of selective media

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A procedure utilizing selective agar media, developed for detecting *F. graminearum* in seed, was tested with cereal crowns. Segments of the crown from 1743 barley plants were placed onto peptone-pentachloronitrobenzene (PCNB) agar, used specifically to isolate *Fusarium* spp. After 7 days, the 1520 colonies of *Fusarium* spp. that developed were transferred to FGA (*F. graminearum* agar). The production of a red zone from the point of inoculation after 7 days indicated the presence of *F. graminearum* in 195 colonies. To validate results, all those which produced a red zone on FGA were transferred to PDA where *F. graminearum* was identified by observing with a microscope. *Fusarium graminearum* was confirmed for 184 colonies, a precision of 94%. The specificity of FGA was demonstrated by observing the reaction of a collection of 14 *Fusarium* species represented by 115 isolates. The FGA media was very specific since only isolates of *F. graminearum* (35 of 35) produced the characteristic red colour. Also, none of the 15 isolates of *F. pseudograminearum* produced a red colour, permitting the separation, without molecular techniques, of this very similar appearing species from *F. graminearum*. The PCNB/FGA method is therefore sufficiently reliable in detecting *F. graminearum* in cereal crowns and likely also in other plant parts.

Mise en évidence du *Fusarium graminearum* dans les collets de céréales par isolement sur milieux de culture sélectifs

Une procédure utilisant des milieux de culture sélectifs, mise au point pour détecter le *F. graminearum* dans les semences, a été testée avec des collets de céréales. On a placé des segments de collet de 1743 plantes d'orge sur un milieu gélosé peptone-pentachloronitrobenzene (PCNB), spécifique pour les *Fusarium* spp. Après 7 jours, les 1520 colonies de *Fusarium* qui s'étaient développées ont été repiquées sur le milieu FGA (*F. graminearum* agar). La production d'une zone rouge à partir du point d'inoculation après 7 jours a révélé la présence du *F. graminearum* chez 195 colonies. Pour valider ce résultat, l'identité du *F. graminearum* a été contrôlée par l'observation au microscope des cultures multipliées sur PDA à partir de toutes celles qui avaient produites des zones rouges sur le milieu FGA. Le *F. graminearum* a été confirmé chez 184 colonies, soit une précision de 94 %. La spécificité du milieu FGA a été démontrée en observant la réaction d'une collection de 14 espèces de *Fusarium* représentées par 115 souches. Le milieu FGA s'est révélé très spécifique puisque seules les souches du *F. graminearum* (35 sur 35) ont produit la couleur recherchée. De plus, aucune des 15 souches du *F. pseudograminearum* n'a produit la couleur rouge, ce qui permet de séparer, sans méthode moléculaire, cette espèce très ressemblante au *F. graminearum*. La méthode PCNB/FGA est donc suffisamment fiable pour être utilisée pour détecter le *F. graminearum* dans les collets de céréales et vraisemblablement toute autre partie végétale.

SESSION IV - TOXICOLOGY

Recent Advances in the Toxicology of *Fusarium* Toxins

Genevieve Bondy, Health Canada

Impact of Changing DON Guidelines on Grain Trade

Tom Nowicki, Canadian Grain Commission

Update on Satellite Mycotoxin Network

Mise à jour sur le réseau des mycotoxines

Marc Savard, Agriculture and Agri-Food Canada

Recent Advances in the Toxicology of *Fusarium* Toxins

Genevieve Bondy

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Crop infection with *Fusarium* produces mycotoxins which have been associated with adverse health effects in animals and humans. Recent interest in the toxicology of these secondary metabolites has focussed on deoxynivalenol (DON, vomitoxin) and fumonisin B1 (FB1). DON has been shown experimentally to modulate mucosal immune function, although the link between immunomodulatory effects in rodents and putative effects on immune function in humans and domestic animals has not been clearly established. In recent studies DON transiently increased the susceptibility of mice to enteric reoviral infection, indicating that the toxin suppresses host responses to pathogens. In contrast, FB1 is a non-genotoxic carcinogen that induces renal and hepatic tumours in rodents. Fumonisin-induced equine leukoencephalomalacia and porcine pulmonary edema have been linked to changes in cardiovascular function that are similar across species. In humans epidemiological evidence points to a relationship between maternal FB1 exposure and neural tube defects in offspring. In support of this observation, an increased incidence of neural tube defects has been observed in pups from inbred LMBc mice exposed to FB1 during gestation. Ongoing research on the effects of DON and FB1 at the cellular and molecular level will help to identify subtle physiological changes that will clarify the impact of long-term or sporadic low dose exposures to these contaminants in foods or feeds.

Impact of Changing DON Guidelines on the Grain Trade

Tom Nowicki

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Today, mycotoxins are considered to be the most important category of food safety issue for both domestic and international grain marketing. *Fusarium* fungi, probably the most prevalent toxin-producing fungi in northern temperate regions, are a major reason for this.

Deoxynivalenol (DON, also known as vomitoxin) is the most prevalent *Fusarium* toxin in cereal grains and has been an ongoing concern for the grain trade for over 25 years. Over the past 5-10 years, the level of attention given to DON has been on the rise. Reasons for increasing attention to DON include the high level of concern over its immunosuppressive and general toxic effects and concern over the dietary intakes of *Fusarium* toxins by risk groups like infants and young children. This attention has translated into an increase in regulations governing maximum levels in commercial shipments. The 1995 edition of the FAO/Who compendium on worldwide regulations for mycotoxins showed only five countries having maximum limits for DON in any type of grain. The 2003 compendium shows regulations in place in 37 countries with maximum limits ranging from 300 to 2000 ppb for raw wheat. Of particular concern is the new set of regulations concerning *Fusarium* toxins that will come into effect in the European Union on July 1, 2006.

DON has been a persistent contaminant in eastern Canadian wheats since the early 1980s. *Fusarium* head blight infection of western Canadian wheats has been an ongoing concern since 1993. DON concentrations in Canadian grains vary from one type of grain to another, from province-to-province and from year-to-year. In the case of wheat, DON levels in vessel loadings also vary with class and grade and levels are usually higher in cargoes shipped out of eastern ports than western ports. For evaluating potential marketing problems associated with *Fusarium* head blight infections, the overall ratio of ppm DON to percent *Fusarium*-damaged kernels (FDK) for new crop survey samples is of particular interest to marketers. These ratios change year-to-year and reflect the variable potential for DON production by FDK.

Regulations pertaining to DON pose many concerns and serious challenges for the grain industry, especially for exports. One of the greatest challenges is management of DON levels within the practical and economic constraints of Canada's bulk grain handling system in order to be able to meet contract specifications and national standards. At present, DON is managed indirectly through the use of FDK tolerances within the grading system. This has served the Canadian grain industry very well to date and is very effective for ensuring low DON levels in the highest grades of Canada Western Red Spring and Canada Western Amber Durum wheats. However, management of DON by way of FDK tolerances is problematic when dealing with lower grades and tight DON standards and reliance on this system alone will prove to be even more challenging as more and more countries enact DON standards. Unfortunately, management alternatives are very limited at present due to the lack of a DON test that can be implemented at the elevator driveway.

The inspection and testing procedures of grain importing countries is a major concern for increased regulation of DON. With sampling generally considered to account for over 90% of the variance of mycotoxin determinations in bulk grains, a sound inspection protocol and representative sampling are critical. The importance of these factors is magnified by the fact that DON does not occur homogeneously throughout a bulk loading and that FDK have the potential to stratify within a shipment. The increasing use of ELISA methods for international trade is also a concern due to the tendency for this type of test to generate higher results than LC and GC based methods.

One of the immediate results of increased regulation of DON has been an increase in requests for inspection and testing of shipments at source. Given the high potential for problems, more attention is now being given to the grade being shipped in order to meet contract specifications and national standards. Testing of shipments into transfer and terminal elevators in order to segregate low DON parcels for some customers is a strong possibility, especially for sales of certain grades.

Update on the Mycotoxin Network

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The Mycotoxin Network has been formed to allow direct interaction and dialog between scientists involved in public research on mycotoxins in Canada. At this time it remains an informal network with no set rules or agenda. All members will be able to access the others through e-mail. The priorities in mycotoxin research have been determined to be decontamination methods, analytical method validation, check samples, toxicology, analysis of retail and raw commodities for DON, and the stabilization and increased productivity of the Agriculture and Agri-Food Canada mycotoxin analysis lab.

Mise à jour sur le réseau des mycotoxines

Le réseau des mycotoxines a été formé pour permettre une interaction directe et un dialogue entre les chercheurs impliqués au Canada dans la recherche publique sur les mycotoxines. A date, il reste un réseau informel sans règlements ou agenda. Tous les membres ont accès aux autres par courriel. Les priorités de la recherche sur les mycotoxines au Canada sont: les méthodes de décontamination, la validation de méthodes analytiques, les échantillons standardisés, la toxicologie, l'analyse des produits commerciaux et de base pour le DON, et la stabilisation et l'augmentation de productivité du laboratoire d'analyse des mycotoxines.

SESSION IV - TOXICOLOGY

43.

Purification of a compound from *Cochliobolus sativus* toxic to *Gibberella zeae* (*Fusarium graminearum*)

Purification chez *Cochliobolus sativus* d'un composé toxique à *Gibberella zeae* (*Fusarium graminearum*)

Rampitsch, C.; Bacala, R.; Tekauz, A.; McCallum, B.

44.

Development of novel bioassay system for FHB molecular interaction

Murakami, J.; Ban, T.

45.

Estrogenic mycotoxins in the environment

Hartmann, N.; Erbs, M.; Forrer, H.-R.; Vogelgsang, S.; Wettstein, F.E.; Bucheli, T.D.

46.

Trichothecene: factor of virulence of *Fusarium* seedling blight and root rot in small grain cereals

Les trichothécènes : facteur de virulence de la fonte des semis et de la pourriture racinaire causé par *Fusarium graminearum* chez les céréales

Hwang, S.F.; Wang, H.; Eudes, F.

47.

Effect of heat and water on DON contamination in barley

Abramson, D.

Poster 43.**Purification of a compound from *Cochliobolus sativus* toxic to *Gibberella zeae* (*Fusarium graminearum*)**

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The presence of *Cochliobolus sativus* in cereal spikes and kernels suppresses the growth of *Fusarium graminearum*. A compound which can inhibit the growth of *F. graminearum in vitro* has been partially purified from *C. sativus*. Initial testing defined the host-range and the optimal strain, growth conditions etc. for toxin production. A bioassay based on visible *F. graminearum* growth inhibition was developed to monitor purification progress. The putative toxic compound which is secreted into liquid media was extracted with petroleum ether and separated into fractions by silica gel thin layer chromatography (TLC). Three spots with activity were observed when TLC plates overlaid with *F. graminearum* as clear zones of growth inhibition. Subsequent purification of the toxin by partition chromatography and HPLC, and preliminary analysis by HPLC, GC-MS and proton NMR indicate that the toxin is a small organic compound. More research is required to purify the toxin to homogeneity and determine its structure.

Keywords: *Cochliobolus sativus*, *Fusarium graminearum*, toxin

Purification chez *Cochliobolus sativus* d'un composé toxique à *Gibberella zeae* (*Fusarium graminearum*)

La présence de *Cochliobolus sativus* sur les épis et les grains des céréales réprime la croissance de *Fusarium graminearum*. Un composé capable d'inhiber la croissance de *F. graminearum in vitro* a été partiellement purifié de *C. sativus*. Les analyses préliminaires ont définis la gamme d'hôtes, et la souche optimale ainsi que les conditions de croissance etc. pour la production de la toxine. Un essai biologique basé sur l'inhibition de croissance de *F. graminearum* a été développé pour contrôler le progrès de la purification. Le composé, considéré comme toxine, a été sécrété dans la culture liquide et a été extrait avec l'éther de pétrole et séparé en parties par chromatographie sur couches minces de silica. Trois points actifs ont été vu comme zones claires quand la plaque chromatographique a été envahi avec *F. graminearum*. De purification ultérieurs de la toxine par la chromatographie et HPLC, et les analyses préliminaires par HPLC, la spectrométrie par chromatographie gazeuse et de masse et la résonance magnétique nucléaire, indiquent tous que la toxine est un composé organique. D'autres recherches sont nécessaire pour purifier la toxine jusqu' l'homogeneity et déterminer sa structure.

Mots clés : *Cochliobolus sativus*, *Fusarium graminearum*, toxine

Poster 44.

Development of novel bioassay system for FHB molecular interaction

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A new bioassay system using the primary leaves of wheat was developed and used to evaluate *Fusarium graminearum* pathogenicity and gene expression *in planta*. This assay could detect the difference of pathogenicity between resistant cv. Sumai 3 and susceptible cv. Gamnanya. When a drop of the conidial suspension was placed on wounded portion of the leaves, the pathogen succeeded in infection and produced an oval lesion. On the other hand, when leaf tissue was treated with purified toxin (DON) water-soaked symptoms were observed. Lesion size was remarkably extended in case of mix treatment with toxin and conidial suspension. These results indicate that this toxin plays an important role in lesion development of this fungus. DON and NIV mycotoxins have been shown to be a virulence factor in FHB and some toxin synthesis genes have already been cloned. However, their role in host-parasite interaction is not fully clear. As such, fungal gene expressions were investigated using total RNA prepared from infection sites on primary leaves. Transcript accumulation of two constitutive genes and two trichothecene genes were detectable by PCR no later than 24 h after inoculation. This suggests that new bioassay systems could be developed to analyze the early infection events by molecular level.

Poster 45.

Estrogenic mycotoxins in the environment

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Zearalenone (ZON) is the most prominent of the resorcyclic acid lactones (RALs) produced by *Fusarium* species (e.g. *F. graminearum*) that grow on corn, wheat, and other cereals. ZON has been known to be a potent natural estrogen for almost half a century, and plenty of effort has been made to understand the occurrence and behaviour of RALs in food, feed, and domestic animals. In contrast, very little is known over the fate and behaviour of RALs in the environment and their impact on ecosystems. The estrogenic activity of RALs is comparable with that of natural estrogens. However, in comparison with many well-known synthetic endocrine disruptors (such as bisphenol A), their estrogenic potencies are several orders of magnitude higher. The physico-chemical properties and the few findings from environmental studies suggest that these compounds are very likely to be emitted into the environment. Toxins released from *Fusarium*-infested plants might enter the soil and infiltrate into groundwater/drainage water and/or elute by surface runoff into surface waters. Our research activities within the Swiss National Research Program 50 'Endocrine Disruptors: Relevance to Humans, Animals, and Ecosystems' are focused on the above indicated pathway. Preliminary results demonstrate that *Fusarium*-infested wheat plants are potential sources of ZON. Following heavy rainfall in surface water pools, up to 250 ng/L were found whereas in drainage water, a few ng/L were detected.

Poster 46.

Trichothecene: factor of virulence of *Fusarium* seedling blight and root rot in small grain cereals

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Previous studies have indicated that trichothecenes influence pathogen virulence in *Fusarium* head blight disease caused by *Fusarium graminearum*. Greenhouse trials were conducted in 2003 and 2004 to investigate the impact of trichothecenes on the severity of seedling blight and root rot in common wheat, durum wheat, barley, and triticale using two trichothecene-producing and two trichothecene-non-producing *F. graminearum* strains. In 2003, seedling emergence and survival for the trichothecene-producing strain (Gz3639) were significantly reduced compared to the trichothecene-non-producing strain (GzT40), while root rot incidence and severity were significantly increased. In 2004, two trichothecene-producing strains (Gz3639 and GzT106) reduced seedling emergence and survival ($P \leq 0.01$) in eight of ten crops/cultivars based on the single degree-of-freedom contrasts. Inoculation with GzT106, a trichothecene-producing dd-back strain, resulted in more severe root rot symptoms in eight of ten cultivars ($P \leq 0.01-0.05$) and lower seedling emergence and survival in seven of ten cultivars ($P \leq 0.01-0.10$) comparing to the wild type parental strain Gz3639. The presence of trichothecenes may play an important role in the virulence of *F. graminearum* as the causal pathogen of root rot and seedling blight in cereals.

Keywords: *Fusarium graminearum*, Tri5+, Tr5-, isogenic strains, trichothecenes; cereals

Les trichothécènes : facteur de virulence de la fonte des semis et de la pourriture racinaire causé par *Fusarium graminearum* chez les céréales

Des études précédentes ont démontré que les trichothécènes produites par *Fusarium graminearum* sont des facteurs de virulence de la fusariose de l'épi chez les céréales. En 2003 et 2004, des essais en serres ont été réalisés pour étudier l'impact des trichothécènes sur la sévérité de la fonte des semis et de la pourriture racinaire chez le blé tendre, le blé dur, l'orge et le triticale. Deux souches de *F. graminearum* ont été utilisées, l'une produisant et l'autre ne produisant pas les trichothécènes. En 2003, l'émergence et la survie des plantules inoculées avec la souche productrice de trichothécènes (Gz3639) étaient significativement réduites comparées à celles observées sous inoculation avec la souche non productrice de trichothécènes (GzT40), alors que l'incidence et la sévérité de la pourriture racinaire étaient accrues. En 2004, deux souches productrices de trichothécènes (Gz3639 et GzT106) ont réduit l'émergence et la survie des plantules ($P \leq 0,01$) chez huit sur dix lignées, selon l'analyse des contrastes. L'inoculation avec la souche GzT106, issue de la mutation inverse du gène Tri5 de GzT33, a entraîné davantage de symptômes chez huit sur dix cultivars ($P \leq 0,01-0,05$) et réduit l'émergence et la survie des plantules chez sept cultivars ($P \leq 0,01-0,10$) par rapport à l'inoculation avec la souche sauvage Gz3639. La présence de trichothécènes joue un rôle important sur la virulence de *F. graminearum* dans le cadre de la pourriture racinaire et de la fonte des semis des céréales.

Mots-clés : *Fusarium graminearum*, Tri5+, Tr5-, souches isogéniques, trichothécènes; céréales

Poster 47.

Effect of heat and water on DON contamination in barley

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Canadian barley containing either 18.4 or 4.3 µg/g deoxynivalenol (DON) was heated at 80EC, with small amounts of water or 1M sodium carbonate solution to study the rate of DON reduction. In the 18.4 µg/g DON barley, rapid reductions were observed: with no solutions added, DON declined to 14.7 µg/g after 1 day, and to 4.9 µg/g after 8 days solely due to heat; with water at 10 mL/100 g barley, DON levels reached 3.7 µg/g after 8 days; with 1M sodium carbonate solution added at 10 mL/100 g barley, DON declined to 4.7 µg/g after 1 day, and to 0.4 µg/g after 8 days; with 20 mL/100 g barley, DON declined to 1.4 µg/g after 1 day and to near-zero levels after 8 days. In the 4.3 µg/g DON barley, more gradual reductions were evident: with no solutions added, DON declined to 2.9 µg/g after 8 days solely due to heat; with water at 10 mL/100 g barley, DON levels reached 2.3 µg/g after 8 days; with 1M sodium carbonate solution added at 10 mL/100 g barley, DON declined to 2.7 µg/g after 1 day, and to near-zero levels after 8 days; with 20 mL/100 g barley, DON declined to 1.4 µg/g after 1 day and to near-zero levels after 3, 5 and 8 days.

SESSION V - DISEASE MANAGEMENT AND AGRONOMY

48.

Effect of preceding crop and nitrogen fertilization on DON content in barley

Effet du précédent cultural et de la fertilisation azotée sur le contenu en déoxynivalénol (DON) chez l'orge

Pageau, D.; Lafond, J.; Lajeunesse, J.; Savard, M.E.

49.

Effect of harvesting date and cultivar on DON content in barley

Effet de la date de récolte et du choix du cultivar sur le contenu en DON de l'orge

Lajeunesse, J.; Pageau, D.; Savard, M.E.

50.

Relative pathogenicity of 3-ADON and 15-ADON isolates of *Fusarium graminearum* from the prairie provinces of Canada

Gilbert, J.; Clear, R.M.; Ward, T.J.

51.

***Fusarium* head blight and mycotoxins in cereals - potential strategies to control contamination under conservation tillage**

Vogelgsang, S.; Forrer, H.-R..

52.

***Fusarium* head blight survey of winter wheat in Ontario in 2004**

Tamburic-Ilincic, L.; Schaafsma, A.W.; Xue, A.G.; Chen, Y.; Tenuta, A.

53.

Evaluation of bioagents for the control of fusarium head blight in wheat

Tian, X.L.; Xue, A.G.; Voldeng, H.D.; Chan, Y.-K.; Vigier, B.; Chen, Y.

Poster 48.**Effect of preceding crop and nitrogen fertilization on DON content in barley**

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Since 2001, in the Saguenay-Lac-Saint-Jean area (Quebec), *Fusarium* head blight (FHB) has become a major problem for many barley producers. The effect of four preceding crops (barley, pea, red clover and soybean) and four nitrogen rates (0, 40, 80 and 120 kg ha⁻¹) on DON content in barley were evaluated from 2002 to 2004. In 2002, DON content was significantly lower when barley followed soybean, red clover or pea compared to barley following barley. In 2003, DON content was significantly lower when the preceding crop was pea. However, soybean or red clover as a preceding crop tends also to decrease deoxynivalenol content in barley. In 2004, 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 three years.

Effet du précédent cultural et de la fertilisation azotée sur le contenu en déoxynivalénol (DON) chez l'orge

Depuis 2001, la fusariose de l'épi dans la région du Saguenay-Lac-Saint-Jean (Québec), est devenu un problème important pour les producteurs d'orge. L'effet de quatre précédents culturaux (orge, pois, trèfle rouge et soya) et de quatre fertilisations azotées (0, 40, 80 et 120 kg ha⁻¹) sur le contenu en déoxynivalénol (DON) de l'orge ont été évalués de 2002 à 2004. En 2002, le contenu en DON a été significativement moins élevé lorsque l'orge était ensemencée après le soya, le trèfle rouge ou le pois comparativement à un précédent d'orge. En 2003, le contenu en DON a été significativement plus faible lorsque le précédent cultural était le pois. Cependant, un précédent cultural de soya ou de trèfle rouge avait tendance à réduire le contenu en DON. En 2004, le précédent cultural n'a pas eu d'effet significatif sur le contenu en DON de l'orge. L'effet de la fertilisation azotée sur le contenu en DON n'a pas été significatif au cours des trois années.

Poster 49.

Effect of harvesting date and cultivar on DON content in barley

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In the Saguenay-Lac-Saint-Jean area (Quebec, Canada), *Fusarium* head blight has become a major problem in barley (*Hordeum vulgare* L.). In 2003 and 2004, a trial was conducted at the Research Farm of Agriculture and Agri-Food Canada in Normandin to determine the effect of harvesting date and barley cultivar on deoxynivalenol (DON) content in grain. Three barley cultivars (AC Klinck, Brucefield, and Viviane) and three harvesting dates were evaluated in a factorial randomized block design with four replicates. For both years, the interaction Harvesting date X Cultivar was not significant. Also, the harvesting date had no significant effect on DON content. In 2003 and 2004, DON content of cultivar AC Klinck was significantly lower than the other cultivars. Moreover, in 2004, DON content of the cultivar Viviane was significantly lower than Brucefield. The results indicate that delaying harvest had no significant effect on DON content. Nevertheless, the effect of cultivar was significant.

Effet de la date de récolte et du choix du cultivar sur le contenu en DON de l'orge

Dans la région du Saguenay-Lac-Saint-Jean (Québec, Canada), la *fusariose* de l'épi est devenu un problème important dans la culture d'orge (*Hordeum vulgare* L.). En 2003 et 2004, une expérience factorielle, comprenant 4 répétitions, a été menée à la ferme de recherches d'Agriculture et Agroalimentaire Canada à Normandin afin de déterminer l'effet de la date de récolte et du choix du cultivar d'orge sur le contenu en déoxynivalénol (DON) dans le grain. Trois cultivars d'orge (AC Klinck, Brucefield, et Viviane) et trois dates de récolte ont été évalués. Au cours des deux années, l'interaction Date de récolte X Cultivar n'était pas significative. De plus, la date de récolte n'a eu aucun effet significatif sur le contenu en DON du grain. En 2003 et 2004, le contenu en DON du cultivar AC Klinck était significativement plus faible que celui des autres cultivars. Aussi, en 2004, le cultivar Viviane avait un contenu en DON plus faible que Brucefield. Les résultats ont démontré que le contenu en DON du grain d'orge n'était pas affecté par la date de récolte. Cependant, l'effet du choix du cultivar était significatif.

Poster 50.

Relative pathogenicity of 3-ADON and 15-ADON isolates of *Fusarium graminearum* from the prairie provinces of Canada.

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Eighteen isolates of *Fusarium graminearum* Schwabe, 3 producing 15-ADON and 3 producing 3-ADON from each of the Canadian provinces of Manitoba, Saskatchewan and Alberta, were tested for relative pathogenicity and consistency of production of toxin, on two Canadian spring wheat cultivars, 'Roblin' (S) and '5602 HR' (MR). The experimental design was a 3-replicate randomized complete block. Each replicate consisted of a pot containing 2 or 3 plants of one cultivar, and were grown in a cooled greenhouse. At anthesis, 2 to 5 heads per pot were inoculated with one isolate and heads covered with glassine bags for 48 h to promote a favourable environment for disease development. Disease was scored at 14 d and 21 d after inoculation and recorded as percentage infected spikelets. These preliminary results showed no significant differences in pathogenicity among isolates from the three provinces and producing either 3-ADON or 15-ADON. Toxin analysis by GC/MS of seeds from the inoculated heads found higher levels of DON in the susceptible (45 ppm) vs resistant (14 ppm) wheat. All isolates formed their respective 3-ADON or 15-ADON analogs. The 3-ADON isolates formed, on average, 16.1 ppm DON on 5602HR and 56.9 ppm on Roblin, higher than the 15-ADON isolates which averaged 11.9 ppm and 33.6 ppm respectively.

Poster 51.

***Fusarium* head blight and mycotoxins in cereals - potential strategies to control contamination under conservation tillage**

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The occurrence of *Fusarium* head blight (FHB) in cereals is strongly influenced by cultivation practices such as crop rotation, tillage, and choice of varieties. When wheat is grown after corn and especially with corn residues remaining on the soil surface (i.e. reduced or zero tillage), FHB caused by *Fusarium graminearum* (FG) and contamination of wheat with one of its associated toxins, deoxynivalenol, is more prevalent. If these two risk factors are combined, serious mycotoxin contaminations can occur even with the most resistant varieties currently grown. In order to avoid FHB and mycotoxins while protecting the soil with conservation tillage, combinations of several measures have to be developed. We assume that with an accelerated decomposition of crop debris as the main source of overwintering *Fusarium* inoculum, the risk for infection would be reduced. Since 2003, we investigate the effect of straw management in on-farm trials with zero tillage in a corn-wheat rotation. On 4 sites, we examined the effect of fine mulching with or without surface incorporation towards the occurrence of FHB. First results show that with a less susceptible wheat variety and fine chopping of corn straw it is possible to produce no-till wheat with low DON contents. However, inconsistent results between different trial locations demonstrate that further research is needed. We suppose that the differing results are primarily due to differences in mulch quality such as the size of mulched debris and the homogeneity of dispersal, aspects we will pay more attention to in the future. The results of this research project focussing on straw management and decomposition will be important for both no-till and reduced tillage systems. Furthermore, it will contribute towards safe food and feed while respecting the environment.

Poster 52.

***Fusarium* head blight survey of winter wheat in Ontario in 2004**

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Fusarium head blight (FHB) caused significant losses in winter wheat in Ontario in 2004, the third recent FHB epidemic year, following the outbreaks in 2000 and 1996. A survey was conducted in commercial winter wheat fields selected at random. The FHB index was calculated as incidence X severity/100. Deoxynivalenol (DON) level was determined using a quantitative fluorometric test-FluoroQuan (Romer Labs™, Inc, Union, MO). The number of healthy and *Fusarium* damaged kernels (FDK) were counted and % FDK was calculated. The kernels were also sterilized, plated on the selective medium and *Fusarium* species were identified using microscopic examination. The FHB index, DON level, % FDK and % of *F. graminearum* infected seeds ranged from 1.0-43.7%, 0-9.7 ppm, 0.2-6.0% and from 0-45%, respectively. *F. graminearum* was the predominant species isolated. Other species isolated from the infected kernels included: *F. acuminatum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides*. However, these were isolated infrequently, each occurring on less than 1% of kernels. We expect that losses would have been greater in the absence of: tebuconazole (FOLICUR™) application, the use of moderately resistant cultivars (Wonder, Vienna, AC Morley, Platinum, Wisdom) and agronomic practices recommended for FHB management.

Poster 53.

Evaluation of bioagents for the control of *Fusarium* head blight in wheat

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Six bioagents including three bacteria (*Bacillus subtilis* [Kodiak], the culture filtrate of *Bacillus subtilis* [D1/2], and *Streptomyces griseoviridis* [Mycostop]) and three fungi (*Clonostachys rosea* [ACM941], *Penicillium bilaii* [Jumpstart], and *Trichoderma* sp.) were evaluated for their antagonistic effectiveness against *Fusarium graminearum* Schwabe, the causal agent of fusarium head blight (FHB) of wheat. In laboratory tests, all the bioagents significantly inhibited the growth of the pathogen in dual cultures. ACM941, Mycostop and D1/2 completely inhibited macrospore germination of *F. graminearum* when co-inoculated with the pathogen for six hours while *Trichoderma* sp. and Kodiak treatments had 5.2 and 18.1 % of spore germination, respectively. However, Jumpstart treatment allowed >90% spore germination, which was not significantly different from the control. Field trials were conducted in an FHB nursery at the Central Experimental Farm, Ottawa, Ontario, in 2005. When *F. graminearum* infected barley, corn kernels and wheat debris were treated with the bioagents and placed in the nursery, the number of perithecia developed on kernels and debris were significantly reduced by ACM941 and *Trichoderma* sp. but not by the other treatments, compared to the untreated control. When applying the bioagents to plants 2-3 times around anthesis, only ACM941 and *Trichoderma* sp. significantly reduced the FHB disease severity and percentage of *Fusarium*-damaged kernels. Other bioagents showed no significant control of FHB in the field. The effects of ACM941 and *Trichoderma* sp. in reducing perithecium production and control of FHB were less than but not significantly different from those of Folicur (tebuconazole) fungicide used as positive control of such field trials. These results suggest that ACM941 and *Trichoderma* sp. may have potential for controlling FHB in wheat.

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