FINAL PROGRAM
&
ABSTRACTS OF LECTURES AND POSTERS

Joint meeting of the 9th conference
of The World Mycotoxin Forum® and
the XIVth IUPAC International Symposium on Mycotoxins
WMFmeetsIUPAC

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Dr. Isabelle Oswald INRA, France
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Prof.dr. Trevor K. Smith University of Guelph, Canada
Dr. Michele Suman Barilla, Italy
Dr. Mark Sumarah Agriculture and Agri-Food Canada, Canada
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Dr. Mary Torrence U.S. Food and Drug Administration, USA
Dr. Angelika Tritscher World Health Organization, Switzerland
Frans Verstraete, M.Sc. EC-DG Health and Food Safety, Belgium
Dr. Stefan Wagener Canadian Grain Commission, Canada

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Prof.dr. Rudolf Krška BOKU Vienna, Department IFA-Tulln, Austria
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WELCOME

Welcome at WMFmeetsIUPAC, the unique event combining the 9th Conference of The World Mycotoxin Forum® and the XIVth IUPAC International Symposium on Mycotoxins. The conference will build on the success of the previous edition held in Rotterdam, the Netherlands (2012).

The aim of WMFmeetsIUPAC – the world's largest mycotoxin event – is to increase the awareness of human and animal health risks due to mycotoxin contamination. It offers a platform for the food and feed industry, science and regulatory authorities to exchange current knowledge, to promote harmonization of food and feed safety regulations and control procedures, and to make recommendations for integrated strategies ensuring the safety and security of food and feed supply chains.

WMFmeetsIUPAC offers an excellent way to network and to share ideas, providing a reference source for anyone involved in this field. The event will include:

- presentations and discussions in plenary meetings and parallel sessions
- spotlight presentations, case studies and industry updates
- poster sessions
- an instrument/manufacturers exhibition

High-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are values WMFmeetsIUPAC wants to uphold. You are invited to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

Hans P. van Egmond
Rudolf Kraska
Helena B. Bastiaanse

ABOUT THE WORLD MYCOTOXIN FORUM®
The World Mycotoxin Forum® is the leading international meeting series on mycotoxins where food and feed industry representatives meet with people from universities and governments from around the world.

ABOUT IUPAC
The International Union of Pure and Applied Chemistry (IUPAC) – a scientific, international, non-governmental and objective body – serves to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of humankind.
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Key to the abstracts of lectures and posters:
- abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily program;
- the posters are grouped according to theme and then in an alphabetical order according to the corresponding author.

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CANADIAN MUSEUM FOR HUMAN RIGHTS
VISIT AND CONFERENCE DINNER

Wednesday, June 8, 2016
19:30 – 21:00  Visit to the museum
21:00 – 23:00  Conference dinner

Where:
Canadian Museum for Human Rights
85 Israel Asper Way
Winnipeg, MB R3C 0L5

As a special end to the 3rd day of WMFmeetsIUPAC there will be a unique dinner in the Canadian Museum for Human Rights

The Museum's building itself is a 'wow' experience. The unique architecture designed by Antoine Predock parallels a human rights journey – it requires some effort and has a few twists and turns, but can be very rewarding to complete.

Before dinner, you will have ample time to visit the Museum. You will enter the Museum at ground level, into a space that has a subterranean feel. You gradually work your way higher on a series of inclined ramps that reveal more and more daylight. Keep your camera ready – natural light combined with lit, alabaster-covered ramps is always a favorite for photographers.

From the RBC Convention Centre, it is just a 10-minutes walk along York Avenue to the Canadian Museum for Human Rights. Shuttle buses will also run from the Convention Centre to the Museum starting at 19:15, and back from the Museum to the Convention Centre starting at 23:00.

IMPORTANT NOTE

The visit and dinner are only open to participants who registered in advance when they booked for the conference. You have found your ticket for the museum and the conference dinner at the back of your badge that you have received upon arrival at the conference.
## PROGRAM AT A GLANCE

### Monday, June 6, 2016

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>13:45</td>
<td>Plenary meeting</td>
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<tr>
<td>13:45</td>
<td><strong>Mycotoxins in a changing world</strong></td>
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<tr>
<td>17:30</td>
<td>Welcome reception sponsored by R-Biopharm</td>
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### Tuesday, June 7, 2016

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<tr>
<td>08:30</td>
<td>Parallel session 1</td>
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<tr>
<td>08:30</td>
<td><strong>Regulations, economics and trade</strong></td>
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<td>12:30</td>
<td>Lunch break</td>
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<td>14:00</td>
<td>Parallel session 3</td>
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<tr>
<td>14:00</td>
<td><strong>Changing trends in occurrence</strong></td>
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<tr>
<td>18:30</td>
<td>Poster viewing</td>
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<tr>
<td>18:30</td>
<td>Wine tasting sponsored by Biomin and Romer Labs</td>
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### Wednesday, June 8, 2016

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<tr>
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<td>Parallel session 6</td>
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<tr>
<td>08:30</td>
<td><strong>Sampling</strong></td>
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<td>12:30</td>
<td>Lunch break</td>
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<tr>
<td>14:00</td>
<td>Parallel session 8</td>
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<tr>
<td>14:00</td>
<td><strong>Novel techniques in mycotoxin analysis</strong></td>
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<tr>
<td>19:30</td>
<td>Visit to the Canadian Museum for Human Rights Conference dinner</td>
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### Thursday, June 9, 2016

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<tr>
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<td>Parallel session 10</td>
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<tr>
<td>08:30</td>
<td><strong>EmTOX, a research network on emerging mycotoxins</strong></td>
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<tr>
<td>12:30</td>
<td>Lunch break</td>
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<tr>
<td>13:30</td>
<td>Parallel session 12</td>
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<tr>
<td>13:30</td>
<td><strong>Omics of fungi-plant-environment interaction</strong></td>
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<tr>
<td>16:00</td>
<td>Plenary meeting</td>
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<td>16:00</td>
<td><strong>Challenges and opportunities ahead</strong></td>
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### Friday, June 10, 2016

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<tr>
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<td>Excursion (optional)</td>
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CONFERENCE PROGRAM

MONDAY, JUNE 6, 2016

PLENARY MEETING: MYCOTOXINS IN A CHANGING WORLD

13:45 Opening speech
Ms. Leloni Scott, Assistant Deputy Minister, Agri-Industry Development and Advancement Division, Manitoba Agriculture, Food and Rural Development, Canada

14.00 Challenges and opportunities
Jim Smolik, Chief Commissioner, Canadian Grain Commission, Canada

14.10 Welcome to Winnipeg and WMFmeetsIUPAC
Dr. Sheryl Tittlemier, Canadian Grain Commission, Canada

14:20 Introduction and overview of WMFmeetsIUPAC
Prof.dr. Rudolf Krksa, Department IFA-Tulln, BOKU Vienna, Austria
Hans van Egmond, M.Sc., retired from RIKILT Wageningen UR, the Netherlands

14:40 IUPAC – 100 years of creating the common language of chemistry
Dr. Laura McConnell, President Division VI, International Union of Pure and Applied Chemistry (IUPAC) and Bayer CropScience, USA

15:00 Networking break & exhibition

15:30 Mycotoxins in food and feed: an FDA perspective in a changing world
Dr. Nega Beru, U.S. Food and Drug Administration, USA

16:00 EFSA risk assessments on mycotoxins in a changing world
Dr. Mari Eskola, European Food Safety Authority (EFSA), Italy

16:30 Perspectives on FAO’s work on mycotoxins in a changing world
Dr. Vittorio Fattori, Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Italy

17:00 Mycotoxin testing in a changing world: trends in mycotoxin analysis
Ronald Niemeijer, M.Sc., R-Biopharm, Germany

17:30 WELCOME RECEPTION SPONSORED BY R-BIOPHARM

Where:
Delta Grand Ballroom B
Delta Winnipeg Hotel
350 St. Mary Avenue
Winnipeg, MB R3C 3J2

Attendees can take the skywalk from the RBC Convention Centre to the Delta Winnipeg Hotel.
TUESDAY, JUNE 7, 2016

PARALLEL SESSION 1: REGULATIONS, ECONOMICS AND TRADE

Chairs: Dr. Mary Torrence, U.S. Food and Drug Administration, USA
Frans Verstraete, M.Sc., European Commission-Directorate General Health and Food Safety, Belgium

08:30 Chair’s introduction
Dr. Mary Torrence

08:40 A North American perspective on deoxynivalenol management in grains considering new Codex guidelines
Dr. Andreia Bianchini, University of Nebraska-Lincoln, USA

09:05 Economic impact of aflatoxins – situational analysis in six African countries
Dr. Amare Ayalew, African Union Commission, Ethiopia

09:25 Economic impact of mycotoxin contamination in U.S. food and feed production
Dr. Peter Vardon and Dr. Aliya Sassi, U.S. Food and Drug Administration, USA

09:45 The impacts of regulations, economics, and trade on human health
Prof. Dr. Felicia Wu, Michigan State University, USA

10:05 Regulation of mycotoxins in Canada
Luc Pelletier, Health Canada, Canada

10:30 Networking break & exhibition

11:00 Mycotoxins in cereals: regulation and risk management techniques – a Chinese view
Dr. Songxue Wang, Academy of State Administration of Grain, China

11:20 EU regulations and mycotoxins in the food chain: more than a drop in the ocean?
Frans Verstraete, M.Sc., European Commission-Directorate General Health and Food Safety, Belgium

11:40 FDA Food Safety Modernization Act (FSMA) rules: what do they mean for producer and consumer?
Dr. Gerardo Morantes, Cargill, USA

12:00 Regulation of mycotoxins at the Canadian Food Inspection Agency: results from the Feed Mycotoxin Monitoring Program
Dr. Abed Zeibdawi, Canadian Food Inspection Agency, Canada

12:20 Chair’s summary
Frans Verstraete, M.Sc

12:30 Lunch break
Exhibition & poster viewing
TUESDAY, JUNE 7, 2016

PARALLEL SESSION 2: HUMAN AND ANIMAL HEALTH

Chairs: Dr. Genevieve Bondy, Health Canada, Canada  
Dr. Isabelle Oswald, INRA, France

08:30 Chair’s introduction  
Dr. Genevieve Bondy

08:45 Health-based guidance levels for mycotoxins in animal diets: the challenge to identify critical endpoints  
Prof.dr. Johanna Fink-Gremmels, Utrecht University, the Netherlands

09:05 In vivo fate of acetylated and modified trichothecenes in broiler chickens and pigs: a toxicokinetic approach  
Prof.dr. Siska Croubels, Ghent University, Belgium

09:25 Evaluating joint effects of binary mixtures of Penicillium mycotoxins by using bovine macrophage cell line proliferation  
Dr. Niel Karrow, University of Guelph, Canada

09:45 Genome-wide analysis of gene expression: a tool to investigate the toxicity of mycotoxins  
Dr. Imourana Alassane-Kpembi, INRA, France

10:05 New ways of measuring mixtures of mycotoxins cast doubt on conventional regulatory procedures  
Prof.dr. Chris Elliott, Queen’s University Belfast, UK

10:30 Networking break & exhibition

11:00 Combined exposure to multiple mycotoxins in food: are we ready for risk assessment and risk management?  
Dr. Paula Alvito, National Health Institute Doctor Ricardo Jorge, Portugal

11:20 Incidence of acute and chronic co-exposure of fusariotoxins on human hepatocytes  
Dr. Marie-Caroline Smith, Université de Bretagne Occidentale, France

11:40 Biomarkers: aflatoxin exposure and child health  
Dr. Yun Yun Gong, Queen’s University Belfast and University of Leeds, UK

12:00 Human biomonitoring and its application to mycotoxin exposure assessment: revealing the toxicokinetics of deoxynivalenol in humans  
Dr. Ellen Heyndrickx, Ghent University, Belgium

12:20 Chair’s summary  
Dr. Isabelle Oswald

12:30 Lunch break  
Exhibition & poster viewing
TUESDAY, JUNE 7, 2016

PARALLEL SESSION 3: CHANGING TRENDS IN OCCURRENCE

Chairs: Dr. Mark Sumarah, Agriculture and Agri-Food Canada, Canada
        Dr. Amare Ayalew, African Union Commission, Ethiopia

14:00 Chair's introduction
       Dr. Mark Sumarah

14:05 Changing trends in the occurrence of ergot mycotoxins in Western Canada
       Prof.dr. Barry Blakley, University of Saskatchewan, Canada

14:25 Mycotoxins in pet food: the occurrence and significance
       Dr. Larry Thompson, Nestlé Purina Pet Care, USA

14:45 Trends in occurrence of masked mycotoxins in food and feed
       Dr. Franz Berthiller, Department IFA-Tulln, BOKU Vienna, Austria

15:05 Expect the unexpected – common mycotoxins in uncommon places
       Carrie Maune, Trilogy Analytical Laboratory, USA

15:25 Chair's summary
       Dr. Amare Ayalew

15:30 Networking break & exhibition

PARALLEL SESSION 5: SPEED PRESENTATIONS

Chairs: Prof.dr. Hans-Ulrich Humpf, University of Münster, Germany
        Prof.dr. Carlos Mallmann, Federal University of Santa Maria, Brazil

Recently launched projects:

16:00 MyToolBox – Safe food and feed through an integrated (e-)ToolBox for mycotoxin management
       Prof.dr. Rudolf Kriska, Department IFA-Tulln, BOKU Vienna, Austria

16:15 From MycoRed to MycoKey: integrated and innovative key actions for mycotoxin management in the food and feed chain
       Dr. Antonio Logrieco, Institute of Sciences of Food Production, Italy

16:30 The mycotoxin metrology capacity building and knowledge transfer (CBKT) project
       Dr. Ralf Josephs, International Bureau of Weights and Measures, France

Poster presentations:

16:45 Short presentations (7 minutes) by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters
       For details, see page 46-47

18:30 Poster viewing
       WINE TASTING SPONSORED BY BIOMIN AND ROMER LABS
TUESDAY, JUNE 7, 2016

PARALLEL SESSION 4: PRACTICAL SOLUTIONS IN THE SPOTLIGHT

Chairs: Dr. Lei Bao, Nestlé, China

14:00  Mycotoxin analysis in your hand
       Ronald Niemeijer, M.Sc., R-Biopharm, Germany

14:15  The present and the future of mycotoxin risk management
       Dr. Christina Schwab, Biomin, Austria

14:30  Mycotoxin analysis of food and feed: old problems and new challenges require innovative solutions
       Dr. Kurt Brunner, Romer Labs, Austria

14:45  Redefining mycotoxin occurrence and control strategies
       Dr. Alexandros Yiannikouris, Alltech, USA

15:00  Correlation in the occurrence of fusariotoxins in different contaminated feed materials
       Julia Laurain, Olmix, France

15:15  In a global prevention strategy of *Fusarium* mycotoxins, new insights into yeast parietal fractions ability to reduce mycotoxins impact in animal
       Dr. Virginie Marquis, Phileo Lesaffre Animal Care, France

15:30  Networking break & exhibition

16:00  Jump in, the water’s fine – aqueous extraction for mycotoxin rapid tests
       Dr. Stephen Powers, Vicam, A Waters Business, USA

16:15  Sample preparation for LC-MS/MS analysis of mycotoxins
       Dr. Olga Shimelis, MilliporeSigma, USA

16:30  Overcoming matrix interference in the detection of mycotoxins in foods
       Dr. Thu Huynh, Helica Biosystems, USA

16:45  Best practices for rapid mycotoxin testing
       Kyle Donovan, EnviroLogix, USA

17:00  The use of biomarkers to assess efficacy of mycotoxin eliminators *in vivo*
       Dr. Astrid Koppenol, Impextraco, Belgium

17:15  Neogen launches the Reveal Q+ MAX system (mycotoxin aqueous extraction)
       Anthony J. Lupo, Neogen Corporation, USA

17:30  Mycotoxin risk management in the grain supply chain
       Dr. Scarlett Biselli and Mareike Reichel, Eurofins, Germany

17:45  Master-curve calibrated immunoassay for cost-effective detection of mycotoxins
       Maurizio Paleologo Oriundi, Tecna, Italy

18:00  Reliable biochip array technology for the fast, sensitive and semi-quantitative detection of multiple mycotoxins in animal feed
       Janine McMullan, Randox Food Diagnostics, UK

18:15  Smart reader technology and data management for mycotoxin analysis
       Dr. Mark Tess, Charm Sciences, USA

18:30  Poster viewing

WINE TASTING SPONSORED BY BIOMIN AND ROMER LABS
WEDNESDAY, JUNE 8, 2016

PARALLEL SESSION 6: SAMPLING

Chairs: Dr. Sheryl Tittlemier, *Canadian Grain Commission, Canada*  
Dr. Gerardo Morantes, *Cargill, USA*

08:30 Chair's introduction  
Dr. Sheryl Tittlemier

08:35 The importance of sampling in food and feed safety assessment  
Dr. Claudia Paoletti, *European Food Safety Authority (EFSA), Italy*

08:55 Development of an online FAO mycotoxin sampling tool and its application to aflatoxin sampling protocol for corn and groundnuts in Africa  
Dr. Sheryl Tittlemier, *Canadian Grain Commission, Canada*

09.15 Sampling of grain for mycotoxins: understanding the factors that control sampling precision  
Geoffrey Lyman, *Materials Sampling & Consulting, Australia*

09:35 Sampling of cereals – development of a sampling protocol for mycotoxin analysis  
Dr. Brigitte Mahaut, *Arvalis - Institut du Végétal, France*

09:55 Sources of variability in measuring aflatoxin and the role of sampling  
Prof. Dr. Tim Herrman, *Texas A&M University, USA*

10:15 Sampling for mycotoxins in feed – correct heterogeneity characterization  
Dr. Claas Wagner, *Lucerne University of Applied Sciences and Arts, Switzerland and Wagner Consultants, Germany*

10:35 Networking break & exhibition

11:00 Development for aflatoxin sampling plans for Brazil nuts  
Dr. Eugenia A. Vargas, *Ministry of Agriculture, Livestock and Food Supply, Brazil*

11:20 Real-world application and implementation of sampling plans for mycotoxins in pistachios  
Dr. Bob Klein, *Administrative Committee for Pistachios, USA*

11:40 Challenges presented by sampling and low level detection of storage molds in bulk grain  
Dr. Tom Gräfenhan, *Canadian Grain Commission, Canada*

12:00 Which sampling protocol is the most cost-effective?  
Dr. Ine van der Fels-Klerx, *RIKILT Wageningen UR, the Netherlands*

12:20 Chair's summary  
Dr. Gerardo Morantes

12:30 Lunch break  
Exhibition & poster viewing

**COURSE**  
*Representative sampling in practice – the theory of sampling*  
Presenter: Dr. Claas Wagner, *Lucerne University of Applied Sciences and Arts, Switzerland and Wagner Consultants, Germany*  
For details, see page 20
WEDNESDAY, JUNE 8, 2012

PARALLEL SESSION 7: PREVENTION AND CONTROL – PRE-HARVEST

Chairs: Prof.dr. Arthur Schaafsma, University of Guelph, Canada
       Prof.dr. Simon Edwards, Harper Adams University, UK

08:30  Chair’s introduction
       Prof.dr. Arthur Schaafsma

08:40  Pre-harvest management strategies for mycotoxins in cereals in the USA: adapting to change
       Prof.dr. Ruth Dill-Macky, University of Minnesota, USA

09:05  Cropping factors: the key for sustainable mycotoxin management in cereals – a European
       perspective
       Dr. Susanne Vogelgsang, Agroscope, Switzerland

09:30  Recent advances in adoption of aflatoxin biocontrol by farmers in Africa
       Dr. Ranajit Bandyopadhyay, International Institute of Tropical Agriculture, Nigeria

09:50  The role of endophytes in biological control of toxigenic fungi: new hope for success?
       Dr. Manish Raizada, University of Guelph, Canada

10:10  The effect of corn plant density and water availability on fumonisin-producing Fusarium spp.
       infection and fumonisin synthesis
       Dr. Belinda Janse van Rensburg, Agricultural Research Council, South Africa

10:30  Networking break & exhibition

11:00  Ergot management solutions: an integrated approach in Western Canada
       Dr. James Menzies, Agriculture and Agri-Food Canada, Canada

11:20  Cisgenic wheat, through genome editing, to reduce Fusarium mycotoxins in grains
       Dr. Ajjamada Kushalappa, McGill University, Canada

11:40  Support from modeling in climate change scenarios
       Prof. dr. Paola Battilani, Università Cattolica del Sacro Cuore, Italy

12:00  Is organic farming a suitable approach to reduce mycotoxins in agricultural crops?
       Dr. Gunnar S. Eriksen, Norwegian Veterinary Institute, Norway

12:20  Chair’s summary
       Prof.dr. Simon Edwards

12:30  Lunch break
       Exhibition & poster viewing

COURSE

Representative sampling in practice – the theory of sampling
Presenter: Dr. Claas Wagner, Lucerne University of Applied Sciences and Arts, Switzerland
           and Wagner Consultants, Germany
For details, see page 20
PARALLEL SESSION 8: NOVEL TECHNIQUES IN MYCOTOXIN ANALYSIS

Chairs: Dr. Chris Maragos, U.S. Department of Agriculture, USA
       Prof.dr. Sarah De Saeger, Ghent University, Belgium

14:00 Chair’s introduction
       Prof.dr. Sarah De Saeger

14:10 Validation of the planar waveguide biosensor for multimycotoxin analysis of wheat
       Dr. Sheryl Tittlemier, Canadian Grain Commission, Canada

14:30 Dried blood spots: a powerful tool to analyze mycotoxins in blood samples
       Prof.dr. Hans-Ulrich Humpf, University of Münster, Germany

14:50 Progress and challenges in aptamer-based mycotoxin sensing
       Dr. Maria DeRosa, Carleton University, Canada

15:10 Optimization and validation of LC-MS/MS based methods for the simultaneous determination of hundreds of fungal metabolites
       Dr. Michael Sulyok, Department IFA-Tulln, BOKU Vienna, Austria

15:30 Networking break & exhibition

16:00 Safe foods from farm to table: how IR laser technology and chemometrics spot trace level mycotoxins
       Dr. Gregor Kos, McGill University, Canada

16:20 Entering the ppq levels in mycotoxin biomarker analysis
       Dr. Bojan Šarkanj, University of Osijek, Croatia

16:40 Electrochemical immunosensors based on polyanilino-carbon nanotubes and/or palladium telluride quantum dots for fumonisins detection in corn samples: a comparative study
       Dr. Milua Masikini, University of Western Cape, South Africa

17:00 Luminescence-based immunochemical techniques for rapid screening of (multi)mycotoxins in different matrices
       Dr. Natalia Beloglazova, Ghent University, Belgium

17:20 Chair’s summary
       Dr. Chris Maragos

19:30 VISIT TO THE CANADIAN MUSEUM FOR HUMAN RIGHTS & CONFERENCE DINNER
       For details, see page 5
WEDNESDAY, JUNE 8, 2016

PARALLEL SESSION 9: PREVENTION AND CONTROL – POST-HARVEST

Chairs: Dr. Karen Churchill, Cereals Canada, Canada
       Dr. Michele Suman, Barilla Advanced Laboratory Research, Italy

14:00 Chair's introduction
       Dr. Karen Churchill

14:10 Real-time monitoring of stored commodities for better post-harvest mycotoxin management
       Prof.dr. Naresh Magan, Cranfield University, UK

14:30 Sorting of mycotoxin-contaminated products with hyperspectral imaging
       Prof.dr. Habil Kalkan, Suleyman Demirel University, Turkey

14:50 Decontamination and detoxification of mycotoxins in grain to improve feed quality and animal health
       Dr. Natacha Hogan, University of Saskatchewan, Canada

15:10 Corn grain ensiling is a process conducive to fumonisin B1 degradation: which microbial consortia are responsible?
       Dr. Sylvain Chéreau, INRA, France

15:30 Networking break & exhibition

16:00 Distribution of deoxynivalenol in wheat during milling
       Prof.dr. Dojin Ryu, University of Idaho, USA

16:20 Impact of processing techniques on mycotoxin occurrence in food
       Dr. Michele Suman, Barilla Advanced Laboratory Research, Italy

16:40 Promising detoxification strategies to mitigate mycotoxins
       Dr. Isabelle Oswald and Dr. Jean-Denis Bailly, INRA, France

17:00 Management of food industrial technologies reducing mycotoxins while keeping the quality of finished products
       Dr. Johan De Meester, Cargill, Belgium

17:20 Chair's summary
       Dr. Michele Suman

19:30 VISIT TO THE CANADIAN MUSEUM FOR HUMAN RIGHTS & CONFERENCE DINNER
       For details, see page 5
PARALLEL SESSION 10: EMTOX – A RESEARCH NETWORK ON EMERGING MYCOTOXINS OF POTENTIAL IMPORTANCE FOR CANADIAN AGRICULTURE

Chairs: Dr. Keith Seifert, Agriculture and Agri-Food Canada, Canada
Dr. Tom Gräfenhan, Canadian Grain Commission, Canada

08:30 Chair's introduction
Dr. Tom Gräfenhan/Dr. Keith Seifert

08:35 Taxonomy of fungi producing emerging mycotoxins
Dr. Keith Seifert, Agriculture and Agri-Food Canada, Canada

08:55 Non-targeted screening as a method for profiling mycotoxins
Dr. Justin Renaud, Agriculture and Agri-Food Canada, Canada

09:15 Using yeasts to study mycotoxin toxicity and interactions
Dr. Steve Gleddie, Agriculture and Agri-Food Canada, Canada

09:35 Discovery of novel Fusarium secondary metabolites
Dr. Adilah Bahadoor, Agriculture and Agri-Food Canada, Canada

10:55 Fusarium interspecies interactions during infection of durum wheat
Dr. Linda Harris, Agriculture and Agri-Food Canada, Canada

10:15 New fumonisins from Aspergillus niger and A. welwitschia
Dr. Kevin Burgess, Agriculture and Agri-Food Canada, Canada

10:30 Networking break & exhibition

11:00 Genomics and epigenetics of toxin production in Fusarium
Dr. Gopal Subramaniam, Agriculture and Agri-Food Canada, Canada

11:20 An integrated approach to patulin reduction in food
Dr. Rong Cao, Agriculture and Agri-Food Canada and University of Guelph, Canada

11:40 Ergot fungi: a brief review of taxonomy and a preliminary phylogenetic study of Canadian isolates of Claviceps
Dr. Miao Liu, Agriculture and Agri-Food Canada, Canada

12:00 Ergot alkaloids in sclerotia, wheat, and durum on the Canadian prairies
Dr. Sheryl Tittlemier, Canadian Grain Commission, Canada

12:20 Chair's summary
Dr. Tom Gräfenhan/Dr. Keith Seifert

12:30 Lunch break
Exhibition & poster viewing
THURSDAY, JUNE 9, 2016

PARALLEL SESSION 11: ILSI NORTH AMERICA – RISK-BASED ASSESSMENT OF MYCOTOXINS MITIGATION

Chairs: Dr. Mansi Krishan, ILSI North America, USA
       Dr. Paul Hanlon, Abbott Nutrition, USA

08:30 Chair's introduction and session overview
       Dr. Mansi Krishan

08:40 Man vs. Nature: prevalence of mycotoxins and climate change
       Dr. Anne R. Bridges, AACC International, USA

09:00 Avoiding the unavoidable: regional impact and human counter measures to control mycotoxins
       Dr. Amare Ayalew, African Union Commission, Ethiopia

09:20 Global regulatory interventions in the war against mycotoxins
       Dr. Paul South, U.S. Food and Drug Administration, USA

09:40 Impact of mycotoxin regulations on world food trade
       Prof.dr. Felicia Wu, Michigan State University, USA

10:00 Impact of regulatory mycotoxin mitigation efforts
       Prof.dr. Samuel Godefroy, Université Laval, Canada

10:30 Networking break & exhibition

11:00 Introduction of proactive risk-based decision making to mitigate mycotoxin risk
       Dr. Paul Hanlon, Abbott Nutrition, USA

11:25 Use of state-of-the-art exposure assessment for mycotoxins
       Cian O'Mahony, Crème Global, Ireland

11:50 Mycotoxin mitigation as a model for risk-based decision making
       Dr. Joseph. V. Rodricks, Ramboll Environ, USA

12:20 Chair's summary
       Dr. Paul Hanlon

12:30 Lunch break
       Exhibition & poster viewing
THURSDAY, JUNE 9, 2016

PARALLEL SESSION12: OMICS OF THE ORGANIZED COMPLEXITY OF FUNGI-PLANT-ENVIRONMENT INTERACTION IN MYCOTOXIN PRODUCTION

Chairs: Prof.dr. Paola Battilani, *Università Cattolica del Sacro Cuore, Italy*
Dr. Deepak Bhatnagar, *U.S. Department of Agriculture, USA*

13:30 Chair's introduction
Prof.dr. Paola Battilani

13:40 Aflatoxin biosynthesis and the related metabolism(s)
Prof.dr. Kimiko Yabe, *Fukui University of Technology, Japan*

14:00 *Aspergillus flavus* secondary metabolic gene clusters: the good, the bad, and the bashful
Dr. Jeffrey Cary, *U.S. Department of Agriculture, USA*

14:20 Ochratoxin A biosynthetic pathway and its regulatory mechanisms
Prof.dr. Yang Liu, *Chinese Academy of Agricultural Sciences, China*

14:40 Lipid-mediated signaling between fungi and plants govern production of mycotoxins
Prof.dr. Michael Kolomiets, *Texas A&M University, USA*

15:00 Breeding corn for resistance to *Fusarium* ear rot: an integrated approach of transcriptomics, metabolomics and genomics
Dr. Alessandra Lanubile, *Università Cattolica del Sacro Cuore, Italy*

15:20 Chair's summary
Dr. Deepak Bhatnagar

15:30 Networking break & exhibition

PLENARY MEETING: CHALLENGES AND OPPORTUNITIES AHEAD

Chairs: Prof.dr. Rudolf Kriska, *Department IFA-Tulln, BOKU Vienna, Austria*
Hans van Egmond, M.Sc., retired from *RIKILT Wageningen UR, the Netherlands*

For details, see page 19
THURSDAY, JUNE 9, 2016

PARALLEL SESSION 13: THE FUTURE OF MYCOTOXIN DETOXIFICATION STRATEGIES

Chairs: Dr. Gerd Schatzmayr, Biomin Research Center, Austria
Dr. Larry Thompson, Nestlé Purina Pet Care, USA

13:30 Chair’s introduction
Dr. Gerd Schatzmayr

13:35 Mycotoxin binders for the reduction of aflatoxins in animals and humans
Sarah Elmore, M.Sc., Texas A&M University, USA

13:55 Recent advances in microbial and enzymatic detoxification of mycotoxins – epimerization of deoxynivalenol
Dr. Ting Zhou, Agriculture and Agri-Food Canada, Canada

14:15 Novel approaches to assess mycotoxin detoxification methods in feed – biomarkers of exposure and effects
Dr. Heidi Schwartz-Zimmermann, Department IFA-Tulln, BOKU Vienna, Austria

14:35 Round table discussion: Current and future legislation of mycotoxin binding and detoxifying products
Dr. Gerald Schultheis, EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA), Belgium
Frans Verstraeete, M.Sc., European Commission-Directorate General Health and Food Safety, Belgium
Dr. Michael Henry, U.S. Food and Drug Administration, USA
Dr. Jinquan Wang, Feed Research Institute, Chinese Academy of Agricultural Sciences (CAAS), China

15:25 Chair’s summary
Dr. Larry Thompson

15:30 Networking break & exhibition

PLENARY MEETING: CHALLENGES AND OPPORTUNITIES AHEAD

Chairs: Prof.dr. Rudolf Kraska, Department IFA-Tulln, BOKU Vienna, Austria
Hans van Egmond, M.Sc., retired from RIKILT Wageningen UR, the Netherlands

16:00 After 125 years of mycotoxin research, what is expected from researchers now?
Prof.dr. David Miller, Carleton University, Canada

16:30 Presentation of the Best Poster Award

16:40 Top five answers learned at WMFmeetsIUPAC
Prof.dr. Rudolf Kraska and Hans van Egmond, M.Sc.

17:00 Closing of WMFmeetsIUPAC
Course: Representative sampling in practice – the theory of sampling

Samples are extracted for various reasons, using many different sampling procedures in a wide range of application fields addressing a bewildering array of different material types. But what is the meaning of analyzing any sample if it cannot be documented to be representative? The answer is ‘none’!

This short course gives an introduction to the Theory of Sampling (TOS), the only fully comprehensive approach to representative sampling. Rather than focusing on the theoretical aspect of TOS, the course covers the main practicalities of representative sampling, including a set of scale-invariant sampling operations, which allow to tackle the main problem of representative sampling: heterogeneity.

Course presenter:
Dr. Claas Wagner is an international sampling consultant, founder of Wagner consultants and lecturer at the Lucerne University of Applied Sciences and Arts, Switzerland. He has published various peer-reviewed articles on representative sampling and chemometrics and is the editor of the Sampling Column in Spectroscopy Europe/Asia, as well as editor of the proceedings of the 7th World Conference on Sampling and Blending.
As the curtain rose on the 20th century, chemistry was already a mature science and a thriving industry. But communication within the industry was difficult. There were few generally accepted norms for the naming of chemical compounds and chemists routinely did so according to their own personal preferences, resulting in multiple names for a single, unique compound. This lack of a universally accepted language in chemistry created a major barrier to the sharing of information, ultimately hindering efficient research and the rapid advancement of scientific discovery. Aware of this major barrier to the growth of scientific knowledge, a group of pure and applied chemists gathered in 1919 to create the International Union of Pure and Applied Chemistry (IUPAC). IUPAC was given the charter not only to create a nomenclature that would facilitate communication within the chemistry community, but also to develop standards and norms for the calibration and normalization of chemical substances. Today, one hundred years later, IUPAC and the field of chemistry jointly face many interwoven challenges such as globalization, the energy crisis, climate change, and other environmental challenges. The IUPAC Centenary, to be celebrated in 2019, offers an opportunity not only to commemorate this century-old organization, but also to rethink how IUPAC can better promote and advance the evolving field of chemistry. It is an opportune time to take a critical and ambitious look at IUPAC in order to prepare the global chemistry community for the future.

Mycotoxins in food and feed: an FDA perspective in a changing world

Nega Beru
Director, Office of Food Safety, Center for Food Safety and Applied Nutrition, Food and Drug Administration, USA; nega.beru@hhs.fda.gov

The Food and Drug Administration (FDA) is mandated under the U.S. Federal Food, Drug and Cosmetic Act (FFDCA) to control the presence of poisonous or deleterious substances such as mycotoxins in food and feed which may render them harmful to health. Contaminants are specifically regulated under Section 402(a)(1) of the FFDCA. This statute allows the FDA to prohibit the entry of adulterated food into interstate commerce. The general strategies used to enforce the Act in regards to mycotoxins include: (1) conducting science-based safety/risk assessments and analysis; (2) establishing action or guidance levels; (3) monitoring the food and feed supply to ensure compliance with established levels; and (4) initiating appropriate enforcement action when violations occur.

With enactment of the FDA Food Safety Modernization Act (FSMA) in 2011, there has been a major paradigm shift in how FDA approaches food safety. The FSMA shifts FDA’s food-safety focus from reaction and response to prevention of foodborne contamination, so that prudent preventive measures will be systematically built into all parts of the food system. Preventive controls are not new to FDA as FDA requires them for foods such as juices, seafood and low acid canned foods. But FSMA gives FDA an explicit mandate to use the system more broadly. FDA has issued a number of regulations to implement FSMA. For example, the Preventive Controls for Human Food and the Preventive Controls for Food for Animals rules require that makers of food to be consumed in the United States develop a written food safety plan for protecting their food products from contamination, whether that food is produced at a foreign- or domestic-based facility. Specifics of the rule are as follows: (1) facilities covered by the rule must establish and implement a food safety system that includes an analysis of hazards; (2) facilities must have preventive control measures in place to ensure that identified hazards will be prevented or minimized; (3) monitoring processes must be conducted to ensure that the preventive controls are consistently performed; (4) timely corrective actions are taken to identify and correct a preventive control that is not properly implemented; and (5) verification activities are performed to show the food safety plan is properly implemented and effective. Another example is the Foreign Supplier Verification Program (FSVP) rule. Importers covered by the rule must have a program in place to verify that their foreign suppliers are producing food in a manner that provides the same level of public health protection as the preventive controls rules for human and animal food and the new produce safety
standards. FSVP is one mechanism through which FDA can assess compliance with preventive controls by overseas producers.

**EFSA risk assessments on mycotoxins in a changing world**

*Mari Eskola*
Scientific Officer, Team on Contaminants, Unit on Biological Hazards and Contaminants (BIOCONTAM), European Food Safety Authority (EFSA), Italy; mari.eskola@efsa.europa.eu

European Food Safety Authority (EFSA) carries out risk assessments on food and feed safety at the European level. In the European food safety system, risk assessment is undertaken independently from risk management. As the risk assessor, EFSA produces scientific opinions and advice as the basis of a sound foundation for European policies and legislation. Thus EFSA supports the European Commission, European Parliament and European Union Member States in their risk management decisions. EFSA’s remit is wide and covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. EFSA’s Scientific Panels and Committee have crucial roles in developing its scientific opinions. The experts of the Scientific Panels and Committee come from all over Europe and the world to contribute to the scientific opinions. The EFSA Panel on Contaminants in the food chain (CONTAM Panel) does risk assessments in the area of chemical contaminants in food and feed, namely process contaminants, environmental contaminants, natural toxicants, mycotoxins and residues of unauthorized substances.

Mycotoxins have constituted a main work area for the CONTAM Panel since 2004; with 22 scientific opinions on the risks to human and/or animal health related to the presence of mycotoxins in food and/or feed being released by the Panel. Most recently modified mycotoxins (known also masked mycotoxins) and the health risk they may pose to humans and animals were included in the assessments. All these risk assessments were all developed upon request from the European Commission.

This presentation gives an overview of the risk assessments on mycotoxins developed by the EFSA CONTAM Panel and discusses the challenges the changing world and environment are bringing to the mycotoxin risk assessments in Europe. In addition, some thoughts are presented on the latest activities of EFSA on mycotoxins with regard to mixtures of mycotoxins, feed additives and plant health.

**Perspectives on FAO’s work on mycotoxins in a changing world**

*Vittorio Fattori*
Food Safety and Quality Unit, Food and Agriculture Organization of the United Nations (FAO), Italy; vittorio.fattori@fao.org

The importance of public health challenges as well as trade issues posed by mycotoxins contamination of food commodities, have made through the years prevention and control of these toxins an important area of work of the food safety program of the Food and Agriculture Organization of the United Nations (FAO). Addressing mycotoxins is indeed closely linked to the FAO’s mandate, as the very nature of their contamination, affecting staples food such as corn, sorghum etc. has important food security implications for many developing countries’ populations. In a rapidly changing world, the food safety context is also in constant evolution, and as such it is important to understand and be prepared to the new and emerging challenges in the area of mycotoxins. Climate change, for instance, is likely to lead to increased occurrence of aflatoxins and other mycotoxins (and possibly their increased co-occurrence) in many countries just as new technologies may prove effective in contributing to future efforts for mycotoxins control.

For many years, FAO in collaboration with other partners like the World Health Organization (WHO) has been carrying out a range of activities aimed at protecting public health, reducing food losses and minimizing the negative economic consequences of mycotoxin contaminations. The work of the Joint FAO/WHO expert Committee on Food Additives and Contaminants (JECFA) has been and continues to be instrumental in allowing Codex Standards to be developed as the foundation for harmonized national sanitary regulations that are based on sound science. However, the existence of sound standards is not sufficient: it is essential that countries have the capacities to implement these standards. This requires commitment of high-level decision-makers to provide the resources and policy environment necessary to support programs of mycotoxin prevention and control. It also requires the
scientific and technical expertise to ensure that programs are well-designed, implemented and their impact monitored. Without coherent and sustained commitment to food safety, mycotoxin contamination cannot be effectively controlled. It is therefore important to:

- raise awareness among policy/decision makers for broad international commitment to the work of Codex;
- strengthen developing countries’ capacities for evidence-based food safety decision-making, including the application of the risk analysis framework;
- assess the applicability of emerging technologies to achieving better prevention and control;
- promote research to better understand threats posed by co-exposure to different mycotoxins;
- promote the sharing of experiences on mycotoxin control in different countries; and
- ensure there is an effective dialogue and information exchange with key stakeholders, including industry and consumers.

Mycotoxin testing in a changing world: trends in mycotoxin analysis

Ronald Niemeijer
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At the 55th anniversary of mycotoxin testing – taking the publication in “Nature” of the famous paper by M.C. Lancaster ‘Toxicity associated with certain samples of groundnuts’ as the beginning of mycotoxin testing – it is a good moment to review how mycotoxin testing developed and which trends in mycotoxin testing currently play a role. Although mycotoxins have plagued mankind since the early days of agricultural production, it wasn’t until the early 60s before one of the many mycotoxins – aflatoxin – was identified and analytical methods were developed. Analytical methods were based on liquid-liquid extraction methods, followed by chromatography – starting with TLC, later by GC and HPLC. In the late 70s and early 80s, antibody-based methods were developed, which started the development of commercial, proprietary methods and test kits. Formats developed rapidly from radioimmunoassay to the methods used frequently nowadays, such as ELISA and lateral flow based assays.

Multitoxin methods. A major breakthrough was the development of the tandem mass spectrometry-based methods at the beginning of this century. In a single run, multiple mycotoxins could be detected. At the same time, these methods have proven to work very well with no or limited sample cleanup. ‘Dilute-and-shoot’- or ‘QuEChERS’-based methods work for many commodities, yet not for all. For those more difficult sample matrices, multitoxin extractions methods based on antibody technology or solid phase extraction, including MIPs were developed.

Analytical efficiency. With more and more different samples to be tested routinely analytical efficiency in the mycotoxin testing laboratory is getting more important. Automation of the actual analysis is pretty common thanks to auto samplers and ELISA pipetting robots. Less common is automated sample preparation, which is now developing strongly. In the light of harmonization and comparability of results but also in terms of lab efficiency, automated sample preparation followed by automated analytical methods will get more and more important.

On-site testing. Testing as early as possible in the food production chain is always more efficient than relying on end-product testing only. For commodities, such as the major cereals but also peanuts, rapid methods, often based on lateral flow immunoassays were developed and are widely used to enable a rapid on-site decision to accept or reject a shipment. Methods are further developed to become faster, easier. Mycotoxin testing methods are evolving from near-site testing to on-site testing.

Mycotoxins and ‘Big Data’? Predictions of the risk of mycotoxin contaminations in the upcoming harvest have been available online for a couple of years now. These models are mainly based on local weather conditions. Climate changes may be a big challenge for these models though. The use of mobile devices in mycotoxin analysis and sharing the analytical data in the cloud might open entirely new ways of data evaluation and predictive modeling.
A North American perspective on DON management in grains considering new Codex guidelines

Andreia Bianchini
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In agricultural commodities, the occurrence of deoxynivalenol (DON) has been reported all over the world, with levels varying among grain types and years of production. The grain supply chain, including growers, buyers, and end users, have effectively managed DON with strategies to control this issue systematically. The safety of consumers is ensured through use of these management strategies. This presentation will cover the occurrence and management of DON in North America, which is accomplished by: (1) a review of publically available data and introduction of new information regarding the occurrence of DON in wheat, corn, and barley in North America, including variability due to growing regions, grain varieties, and year of production; (2) an overview of industry practices to reduce DON contamination from field through milling when necessary; (3) a review of how all in the value chain, including growers, buyers, and end users, have effectively managed DON for more than 20 years; and (4) the potential economic impact of any changes in international regulations.

Economic impact of aflatoxins – situational analysis in six African countries

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Aflatoxin is a complex problem and control is knowledge intensive. Developing strong evidence base to inform policies, interventions and institutional innovations is key for effective aflatoxin control. The Partnership for Aflatoxin Control in Africa (PACA) worked with six countries in Africa to generate locally relevant evidence on the extent of the aflatoxin problem and context-appropriate mitigation measures. This paper highlights results of the situational analysis focusing on the economic impacts of aflatoxins in Gambia, Malawi, Nigeria, Senegal, Tanzania and Uganda. Estimates from the health economics study show the following trends in the countries: Tanzania has estimated that about 3,333 cases of hepatocellular carcinoma (HCC) resulting from aflatoxin exposure would develop, of which an estimated 95% (3,166 persons) will die each year from the disease. These deaths lead to a loss of 96,604 DALYs annually, with financial impact (money that will be saved if efforts to reduce aflatoxin exposures are implemented) from such illness and loss of life ranging from $3 million to $196 million annually. Senegal estimates that HCC cases due to aflatoxin consumption will range between 1,057 and 1,477 while the annual DALYs lost amount to a mean of 98,304, with a monetary equivalent of approximately $128 million. Estimates from The Gambia indicated that about 154 cases of HCC will develop annually due to aflatoxin exposure, resulting to 1,279 Years Lived with Disability (YLD). The monetary value of preventing these losses in the Gambia was estimated at $2.3 million. The overall impact of aflatoxins on the Gambian economy weighs more heavily on human health (53%) than on international trade (47%). Uganda estimated that aflatoxin contamination has an effect on shiling the national economy. These alarming findings demonstrate the urgent need for increased action to mitigate the aflatoxin problem. Dealing with a complex problem, effective aflatoxin control requires systemic thinking for integrated, multi-sectoral, and multi-stakeholder approaches.

Economic impact of mycotoxin contamination in U.S. food and feed production

Peter Vardon, Aliya Sassi and B. Flannery
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Mycotoxins, especially the aflatoxins, the fumonisins, and deoxynivalenol (DON), can be major contaminants of agricultural commodities in the U.S. Mycotoxins cause spoilage and quality deterioration in crops, and reduced health of livestock (from the use of contaminated feed). The combined effects of the feed and food losses reduce the supply and raise the price of various agricultural commodities. Updating Vardon et al. [1], we estimate that the cost of mycotoxin contamination in the U.S. economy is between $2 and $3 billion per year. Data limitations, the variability of commodity price...
and production levels, and the seasonal and geographic variability of contamination levels collude to prevent a more precise annual estimate. We use Monte Carlo computer simulations to estimate the distributions of commodity outputs, prices, and contamination levels. We estimate potential annual costs to be the sum of the value of food losses, feed losses, and mitigation efforts using data from several sources that were not available when Vardon et al. [1] was estimated. Our estimate should be regarded as tentative orders of magnitude calculations, not as final estimates.

References

The impacts of mycotoxin regulations, economics, and trade on human health

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When nations set regulatory standards for food safety, their impacts extend far beyond the national populations they serve. The regulatory limit for mycotoxin standards that one nation sets will have ripple effects on trade worldwide, with potential human health impacts as well. This talk describes some of those impacts, starting with unintended impacts on global food trade, then describing economic impacts to major food exporters, and finally discussing potential impacts to population health. Free trade of food can affect the dietary diversity of populations, which can have multiple beneficial effects, including counteracting the adverse effects of mycotoxins in the diet. A case study is given of how free food trade can have beneficial impacts to public health, of aflatoxin exposure in one region of China.

Regulation of mycotoxins in Canada

Luc Pelletier, E. Elliott and G. Bondy
Food Directorate, Health Products and Food Branch, Health Canada, Canada; luc.pelletier@hc-sc.gc.ca

The Food Directorate of Health Canada is the federal department that is primarily responsible for the regulation of mycotoxins in foods sold in Canada. It establishes policies and standards, and provides advice and information on the safety and nutritional value of food; it also administers the provisions of the Food and Drugs Act and Food and Drug Regulations that relate to public health, safety and nutrition. The Food Directorate conducts toxicological research, generates food residue data, and assesses the human health risks associated with dietary exposure to mycotoxins in Canada. The Canadian Food Inspection Agency (CFIA) conducts regular surveillance for a variety of mycotoxins in the Canadian food supply and is responsible for enforcing the standards established by the Food Directorate. Such enforcement actions may involve recalling products from the Canadian marketplace, if deemed necessary based on the results of the Food Directorate’s health risk assessment. The Canadian Grain Commission (CGC) is responsible for establishing and maintaining quality standards for Canadian grain through the administration of the Canada Grain Act and Regulations. The CGC also conducts research relating to the occurrence and analysis of fungi and associated mycotoxins in raw and processed grain and assesses Canadian grain for compliance with mycotoxin standards, both domestic and international. Health Canada uses a risk analysis framework consistent with that employed by other international regulatory organizations and is also an active participant in international risk assessment and standard setting processes for mycotoxins in foods, in particular those of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Codex Committee on Contaminants in Food (CCCF) and the Codex Alimentarius Commission (CAC).
Mycotoxin in cereals: regulation and risk management techniques – a Chinese view

Songxue Wang
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Mycotoxin contamination is a global problem threatening grain quality and safety. Completely eliminating the mycotoxin-contaminated commodities is still not achievable. China has been paying great attention to mycotoxin contamination problems in grain by setting up corresponding laws, regulations and standards and investing in scientific research and development to enhance the management of grain quality and safety.

China has specified the mycotoxin limits in grain in the ‘Hygienic standard for grains (GB 2715-2005)’ in 2005. These limits have been adopted in the national food safety standards ‘Mycotoxins Limits in Food (GB 2761-2011)’. The new ‘Food Safety Law’ that has been implemented as of October 2015 has for the first time written down the biotoxins into legal documents. Strengthening the grain mycotoxin control has also been called for in the state council document of ‘The Sound Provincial Governor’s Responsibility System for Grain Security (No. 69, 2014)’ released in 2014. To promote the implementation of the ‘Grain Safety Project’, to be specific ‘The Construction Plan of Grain Purchasing, Storage and Supply Security (2015-2020)’, it is required to strengthen the grain and oil quality and safety inspection and monitoring system, to establish a traceable ‘safe grain and seed oil products’ supply platform, to improve the quality and safety standard system, and to enhance the emergency response capabilities, thus to ensure the grain and seed oil quality and safety throughout the whole industry chain. Meanwhile, food safety key technology research and development projects are implemented to provide scientific and technological support.

China has established the risk management concept in grain mycotoxin management shifting from endproduct management to implementing the principle of prevention, which is held essential and with management as supplement. China has established the forecasting of Fusarium head/ear blight system to promote disease prevention, and has built up the early warning system and process control in major grain producing areas. It has been researching risk map development to guide grain purchasing companies to make buying and storage decisions using quick test methods to promote quality control during grain buying and storage to prevent mycotoxin-contaminated grains entering the food and feed market. At the same time, classified storage and utilization based on contaminant levels is under development to enhance the utilization of grains. The risks in each step of the whole chain, from the field, storage, transportation, processing and finally to the consumers, are strictly controlled to minimize the risks to an acceptable level and thus to ensure the grain quality and safety.

EU regulations and mycotoxins in the food chain: more than a drop in the ocean?

Frans Verstraete
European Commission-Directorate General for Health and Food Safety, Belgium; frans.verstraete@ec.europa.eu

The EU has since many years extensive regulations on mycotoxins in feed and food. Prevention is better than cure to protect the humans and animals from the toxic effect of mycotoxins. Therefore, there is a need for encouraging preventive actions, such as good agricultural practice, good storage conditions, and good manufacturing practice. The establishment of maximum limits is not contrary to prevention. The fixing of maximum levels at a reasonably achievable level, stimulates preventive actions at all stages to avoid contamination of the feed/food chain.

In recent years, an increased prevalence and a significant year-to-year variation of the presence of mycotoxins in feed and food in the European region can be observed. Major cause is climate change and in particular the extreme weather conditions during critical growth stages of cereals, in particular corn. However, also other causes (such as agricultural practices) might contribute to the increased prevalence and therefore an in depth root-cause analysis appropriate. The high levels of aflatoxin in the corn harvest 2012 and the high level of Fusarium toxins in the corn harvest 2013 and 2014 have resulted in problems for feed and food supply and safety. This situation entails specific challenges for farmers, feed and food manufacturers, traders and regulators to ensure the safety for animal and human health of feed and food while ensuring the supply of major staple feed and food such as cereals.
To address the mycotoxin challenge, the EU foresees to elaborate a comprehensive EU mycotoxin (prevention) approach, including agricultural and environmental aspects as a sustainable solution.

**FDA Food Safety Modernization Act rules: what do they mean for producers and consumers?**

**Gerardo Morantes**  
Cargill Agricultural Supply Chain, USA; gerardo_morantes@cargill.com

The Food Safety Modernization Act (FSMA) was signed into law by the U.S. president in January 2011. After publication of the proposed rule in October 2013, and a period for public comments where all the important industry trade associations and general public expressed their points of view, and internal reviews to incorporate the industry's feedback, a supplemental rule was published in September 2014. The final rule was published on September 17, 2015. One year from publication, the rule will go into effect (September 2016). Exception to this, will be for 'small businesses' with fewer than 500 full time employees which will have two years, and qualified facilities (very small businesses) with less than $2.5 MYN in sales, adjusted for inflation that will have three additional years to implement the rules. In the U.S., the Food and Drug Administration (FDA) regulates the feed industry. In other words, it has the legal authority to write and enforce regulations and rules that determine how the feed industry operates. The important issue with the new rules is that it is the first time in a seventy-year period that the rules have been updated. It focuses on preventive measures throughout the supply chain, from farm to fork. Therefore, it is perceived as a significant change that could bring increased costs to the business. On the other hand, it may bring a wealth of benefits to the business by ensuring a management system that embraces food safety. There is no question that FSMA brings major changes to how the feed industry will do business in the future. The rule establishes for the first time CGMPs for animal food, comparable to the ones that have long applied to human food. It requires animal food facilities to develop a written food safety plan that includes a hazard analysis and risk based preventive controls.

It is the objective of this presentation, to discuss the most important aspects of the rule, and discuss potential implications for the feed and feed ingredient industries, as well as for the end users of the products being commercialized.

**Regulation of mycotoxins at the Canadian Food Inspection Agency: results from the Feed Mycotoxin Monitoring Program**

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The Canadian Food Inspection Agency (CFIA) is responsible for the regulation of livestock feed, which includes feed safety. Because of the potential negative impact of mycotoxins on human and animal health, the CFIA has the mycotoxin monitoring programs for feed that have been in place for many years under the National Feed Inspection Program. These programs include both random and targeted monitoring for the presence of a number of different mycotoxins that are of concern. Action levels for mycotoxins in livestock feed are used to assess feed compliance, including feed safety. A summary of the data for seven different types of mycotoxins (deoxynivalenol, HT-2 toxin, diacetylscirpenol, T-2 toxin, zearalenone, ochratoxin A, and fumonisins) for feed samples collected from feed mills, farms, and retail stores in Canada from 1990 to 2015, will be presented. The data will be shown in relation to action levels (ALs) for individual mycotoxins and livestock species. The number of samples above and below the ALs will be discussed in relation to livestock species consuming the feed, the feed type tested, sampling area and fiscal year.
Health-based guidance levels for mycotoxins in animal diets: the challenge to identify critical endpoints

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Mycotoxins are currently considered as most prevalent feed contaminants, and recent surveys seem to confirm that farm animals are commonly exposed to diets, containing more than one mycotoxin. Previous approaches to establish safe feed concentrations (acceptable levels) were generally based on the critical assessment of toxicological data related to one single mycotoxin, or a group of very closely related toxins (such as T-2 toxin and HT-2 toxin). Endpoints selected to derive health-based guidance levels for individual animal species included dose-dependent organ-specific effects such as hepatotoxicity, nephrotoxicity, pulmotoxicity or neurotoxicity as well as potential endocrine effects (zearalenone), immunotoxicity and in some cases general observations such as growth retardation. Recent evidence indicates that various mycotoxins, including patulin, fumonisins and trichotheccenes, with deoxynivalenol as most prominent representative, affect directly the intestinal barrier. This alterations in intestinal functions and architecture affects the transport of nutrients, resulting in losses in productivity. Even more importantly, an impairment of the intestinal integrity is followed by an increased intestinal permeability and the transfer of luminal antigens and pathogens to the internal environment, resulting in a local and generalized inflammatory response. The ultimate tolerance of the animal is determined in these cases by multiple factors, not only the actual concentration of a mycotoxin, but also by the presence of other environmental stressors, such as the hygienic quality of the feed, the exposure to pathogens or products thereof like LPS (lipopolysaccharide, a cell wall component of Gram-negative bacteria), and finally the immune competence of the animal. These combined challenges result in an unexpected sensitivity of the animal to common pathogens, a decrease in the response to vaccination programs and an apparently higher susceptibility to long-term exposure to mycotoxins. Analyzing the pathophysiological processes involved in this exposure to multiple stressors, will allow the identification of critical endpoints that could be monitored in animal health programs.

In vivo fate of acetylated and modified trichotheccenes in broiler chickens and pigs: a toxicokinetic approach

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The Fusarium trichotheccenes are frequently detected mycotoxins on a worldwide basis. Besides trichotheccenes as such, food and feed are often co-contaminated with acetylated and modified forms thereof, such as 3-acetyl-deoxynivalenol (3ADON), 15-acetyl-deoxynivalenol (15ADON), deoxynnivalenol-3-β-D-glucoside (DON3G) and T-2 toxin-β-glucoside (T2G). Little is known about the toxicodynamics and toxicokinetics of these acetylated and modified forms. Major questions are: (1) whether in vivo hydrolysis during digestion in mammals and birds results in the release of free mycotoxin, and (2) what is their in vivo fate and disposition in the body. Determination of the oral bioavailability, rate and extent of in vivo hydrolysis, biotransformation and toxicokinetics of 3ADON, 15ADON, DON3G and T2G in relevant animal species may support legislative authorities to assess their risks.

For this purpose, cross-over animal trials were performed with intravenous (IV) and oral (PO) administration of DON, 3ADON and 15ADON to broiler chickens and pigs, and T-2 and T2G to broilers. Plasma concentration-time data were processed via tailor-made compartmental toxicokinetic models. The results in broiler chickens demonstrate that the mean absorbed fraction after PO DON, 3ADON and 15ADON administration was 11, 18 and 42%, respectively. For 3ADON, this fraction was completely hydrolysed presystemically to DON, but for 15ADON it was hydrolyzed to DON to a lesser extent (75%). In pigs, the absorbed fractions were 100% for DON, 3ADON and 15ADON, and 3ADON and 15ADON were completely hydrolyzed presystemically. Also after IV administration, both ADONs were remarkably fast and completely hydrolyzed. When combining these results, both ADONs can be regarded as toxic as DON itself in pigs with respect to systemic toxicity. The results for broilers demonstrate a ‘worst case scenario’ since both ADONs have a higher absorbed fraction, with a four- and twofold higher DON
exposure after 15ADON and 3ADON consumption compared to DON itself, respectively [1]. A similar approach was used for DON3G in broilers and pigs, and for T2G in broilers. Moreover, for DON3G in pigs, the site of hydrolysis was investigated by collecting systemic as well as portal blood. Sampling portal blood offers a valuable tool as it allows to differentiate between presystemic and systemic hydrolysis and therefore contributes to the understanding of the (pre)systemic hydrolysis of modified mycotoxins [2]. Additionally, HRMS analysis of phase II metabolites revealed that DON biotransformation in pigs consists mainly of glucuronidation, and in chickens sulfation predominantly occurs [3]. Phase II biotransformation was much more extensive for chickens than for pigs, which can be a possible explanation for the differences in sensitivity of the latter species to DON.

References

Evaluating joint effects of binary mixtures of *Penicillium* mycotoxin using bovine macrophage cell line

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*Penicillium* mycotoxins (PMs) are common contaminants found in stored silage for animal feed, and these PMs can potentially have joint toxic effects, such as synergism or antagonism, that can affect risk assessment of PM exposure to animals. In the present study, we assessed the joint effect of all possible binary combinations of the following mycotoxins: citrinin (CIT), ochratoxin A (OTA), patulin (PAT), mycophenolic acid (MPA) and penicillic acid (PA), using the independent action (IA) and concentration addition (CA) models. IA and CA models assess toxic interactions of compounds with similar and different modes of action, respectively. Previously published toxicity data (i.e., PM concentration that inhibited the proliferation of a bovine macrophage cell line (BoMacs) by 25 percent, IC25) were initially analyzed, and the IA model determined that OTA+CIT, OTA+PAT and OTA+PA demonstrated synergism (p < 0.05), while PAT+PA and PA+MPA showed antagonism (p < 0.05). The CA model determined that OTA+PA also showed synergism (P<0.05), while PAT+PA also showed antagonism (p < 0.05). When a follow-up more complex experimental design was carried out using binary combinations of PMs at three different dilution levels (i.e., IC25, 0.5*IC25, 0.25*IC25), the results varied depending on PM concentration and the toxicity model. For example, both IA and CA models showed synergism between CIT+OTA at 0.5*IC25 (P<0.05). However, when the IA model was used, binary mixtures of CIT, MPA or PA exhibited antagonism at their IC25s and 0.25*IC25s (P<0.05), PAT+MPA showed antagonism across all tested levels (P<0.05), and PAT+PA showed antagonism at the IC25 (P<0.05) but synergism at the 0.5*IC25 (P<0.05). In contrast, when the CA model was used, binary mixtures of MPA with either OTA or PA showed synergism across all tested levels (P<0.05), OTA+PA and CIT+MPA exhibited synergism at IC25s and 0.25*IC25s (P<0.05), and CIT+PAT showed antagonism at 0.5*IC25 (P<0.05). The present study demonstrates the importance of exposure concentration as well as mycotoxin modes of action when estimating the risk of PM mixture toxicity. This study also provided an informative, efficient, and cost-effective approach to determine interactions between binary mycotoxin mixtures that can be utilized to initially screen mycotoxin interactions before more costly in vivo studies are performed.
Genome-wide analysis of gene expression: a tool to investigate the toxicity of mycotoxins

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Traditionally, toxicology studies have used rodent bioassays to perform the identification and characterization of potentially hazardous substances. It is intended that these bioassays will be the first step, and then different extrapolation methods and safety factors will be applied to the data to generate the health-based guidance values for human and other animal species of interest. The reliability of this approach is frequently questioned since it can lead to incorrect assumptions of hazard to these species of interest. Thus, alternative approaches are needed. The cDNA microarray technology that can provide a broad impression of how organisms respond to stressors by analyzing changes in genome-wide patterns of gene expression may revolutionize the way some toxicological problems are investigated.

We applied the cDNA microarray technology approach to investigate the toxicity of some mycotoxins as well as mycotoxin biotransformation products. The Agilent SurePrint G3 Human GE 8x60K microarray consisting in 62,976 spots was used to compare the toxicity of the apple-contaminating mycotoxin patulin and its biosynthesis precursor ascladiol in human. Likewise, the Agilent porcine specific GPL16524 8x60K microarray consisting in 43,603 spots was used to analyze the intestinal toxicity of type B trichothecenes, including deoxynivalenol (DON), and its biotransformation products DON-3-β-D-glucoside (D3G), deepoxy-DON (DOM-1) and 3-epi-DON (epi-DON) in pig. We could establish the significantly lowered toxicity of ascladiol compared to patulin which resulted in a quite similar gene expression pattern for ascladiol-treated and control conditions. Concerning the B trichothecenes, we demonstrated that de-epoxydation, glucosylation or epimerization of DON decrease its intestinal toxicity, and we defined a global molecular signature of their exposure as well as individual biomarkers that could allow discriminating between them. These findings highlight that the toxicities of type B trichothecenes partially differ each from others, and challenge the current attempts to define toxic equivalency factors for this group of mycotoxins.

New ways of measuring mixtures of mycotoxins cast doubt on conventional regulatory procedures

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Mycotoxins and heavy metals are ubiquitous in the environment and are known to contaminate many types of foods. Coupled to this there is widespread use of pesticides in crop production. Thus there is the potential for many cereal based foods to contain multiple chemical contaminants and compromise their safety to the consumer. A study was undertaken to attempt to identify the severity of the risks, in terms of human exposure, to multiple contaminants. High Content Analysis (HCA) technology was used to measure multiple endpoints to determine cytotoxicity of complex mixtures of mycotoxins, heavy metals and pesticides. The measured endpoints included nuclear intensity (NI), nuclear area (NA), plasma membrane permeability (PMP), mitochondrial membrane potential (MMP) and mitochondrial mass (MM). At concentrations representing EU legal limits of each individual contaminant in corn some of the mixtures were found be cytotoxic for NA and MM endpoints. The most cytotoxic mixture was comprised of 3 mycotoxins, 2 heavy metals and 2 pesticides. The study introduces for the first time, a holistic approach to identify the impact in terms of toxicity to humans when multiple chemical contaminants are present in foods. Governmental regulatory bodies think best how to safeguard the population when such mixtures of contaminants are found in foods and this is an important food safety issue to be addressed.
Combined exposure to multiple mycotoxins in food: are we ready for risk assessment and risk management?

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It is nowadays recognized that the risk of human co-exposure to multiple mycotoxins is real. In the last years, a number of studies have approached the issue of co-exposure and the best way to develop a more precise and realistic assessment. Likewise, the growing concern about the combined effects of mycotoxins and their potential impact on human health has been reflected by the increasing number of toxicological studies on the combined toxicity of these compounds. Nevertheless, risk assessment of these toxins, still follows the conventional paradigm of single exposure and single effects, incorporating only the possibility of additivity but not taking into account the complex dynamics associated to interactions between different mycotoxins or between mycotoxins and other food contaminants. Considering that risk assessment is intimately related to the establishment of regulatory guidelines, once the risk assessment is completed, an effort to reduce or manage the risk should be followed to protect public health. Risk assessment of combined human exposure to multiple mycotoxins thus poses several challenges to scientists, risk assessors and risk managers and opens new avenues for research.

This presentation aims to give an overview of the different challenges posed by the likelihood of human co-exposure to mycotoxins and the possibility of interactive effects occurring after absorption, towards knowledge generation to support a more accurate human risk assessment and risk management. For this purpose, a physiologically-based framework that includes knowledge on the bioaccessibility, toxicokinetics and toxicodynamics of multiple toxins is proposed. Regarding exposure assessment, the need of harmonized food consumption data, availability of multi-analyte methods for mycotoxin quantification, management of left-censored data and use of probabilistic models will be highlighted in order to develop a more precise and realistic exposure assessment. On the other hand, the application of predictive mathematical models to estimate mycotoxins’ combined effects from in vitro toxicity studies will be also discussed. Results from a recent Portuguese project aimed at exploring the toxic effects of mixtures of mycotoxins in infant foods and their potential health impact will be presented as a case study, illustrating the different aspects of risk assessment highlighted in this presentation. Further studies on hazard and exposure assessment of multiple mycotoxins, using harmonized approaches and methodologies, will be crucial towards an improvement in data quality and contributing to holistic risk assessment and risk management strategies for multiple mycotoxins in foodstuffs.

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Incidence of acute and chronic co-exposure of fusariotoxins on human hepatocytes

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Cereals are the most important food and feed resource in the world. Climatic and agricultural practices changes observed over the last years, including the reduction of fungicide use, could lead to food safety problems, especially concerning microbiological contaminations. Among microorganisms contaminating grains, some fungal species (Aspergillus, Fusarium and Penicillium) are toxigenic. In northern temperate regions of the world (America, Asia and Europe), Fusarium spp. are the most problematic species due to their prevalence, ecology, physiology and wide range of mycotoxins (called fusariotoxins) produced. Three fusariotoxins families are particularly important because of their high toxicity and their occurrence in European agricultural products: trichothecenes (mainly deoxynivalenol, nivalenol and T-2 toxin), fumonisins and zearalenone. The severity of the effects depends on the time of exposure, doses and mycotoxin combinations. Fusarium species are able to produce several mycotoxins simultaneously. Moreover, matrices can be simultaneously contaminated by different fungi species. Worldwide, 47% of
the cereal samples analyzed in 2013 were multi-contaminated. However, the risk associated with this multi-contamination is no or a little studied for the moment, whereas there could have implications in various fields including regulatory. Indeed, to this date, mycotoxin regulation has been established for each mycotoxin considered individually. In this context, our main objectives were: (1) to better characterize the risk induced by the simultaneous presence of *Fusarium* toxins in acute and chronic exposure conditions, and (2) to study the cellular mechanisms involved in the response to the exposure to one or more mycotoxins through toxicology and proteomic approaches. For this purpose, we defined 4 fusariotoxin binary mixtures and evaluated their *in vitro* toxicity on the human hepatocyte cell line: HeparG. After 48 h of exposure, IC$_{50}$ for toxin T-2, nivalenol, deoxynivalenol and zearalenone were 0.2, 2.8, 5.9 and 55 µM, respectively. Only IC$_{10}$ were obtained at 10 µM for fumonisin B1 and moniliformin. Co-exposure observed effects were mainly synergistics or additives. Concerning chronic exposure, we selected 3 exposure times (14, 30 and 90 days) and three subtoxic concentrations corresponding to the average exposure dose of French adult population, the tolerable daily intake established by the JECFA and the maximum level permitted in cereals by the European regulation. Effects on viability and cytochrome expression were evaluated. Very few data exist on acute and chronic human mycotoxin co-exposure. Yet, it appears that chronic exposure to multi-contamination in mycotoxin is an important future challenge in risk assessment.

**Biomarkers: aflatoxin exposure and child health**

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Exposure to aflatoxin is prevalent in parts of the developing world due to the consumption of contaminated food, primarily corn and groundnuts. Exposure during the first 1,000 days, a critical age for growth and development, has been shown to have negative effects on child health. Aflatoxin chronic exposure usually coincides with other common problems such as micronutrient deficiencies and infectious disease, which affect the poor and the vulnerable the most. Animal experiments have shown that aflatoxin can reduce growth, inhibit immune function, and exacerbate micronutrient deficiencies. The development and application of biomarkers of exposure have facilitated epidemiological studies of the health effects associated with aflatoxin exposure. Aflatoxin is a known cause for hepatocellular carcinoma, especially when hepatitis B infection is present. There is evidence from several studies associating aflatoxin exposure with stunted child growth, although a causal relationship has yet to be confirmed. A number of biological mechanisms have been proposed. There is also some evidence that immune function, especially cell mediated immunity, may be affected by aflatoxin exposure, but more evidence in humans is required. More recently, there are data accumulating on the co-exposure of aflatoxin with other mycotoxins, which highlights the importance of considering co-exposure in understanding health effects of mycotoxins. The complexity of such co-exposures and possible effects on human health requires the design of sufficiently powerful studies drawing on expertise from both epidemiology and analytical chemistry.

It is vital to understand the burden of aflatoxin exposure on child health in low and middle income countries in order to make timely and effective mitigation decisions. This presentation will review the recent epidemiological evidence for health effects of aflatoxin exposure, including co-exposure with other mycotoxins, and highlight the need for further research in the field. Results from our recent research in The Gambia will be presented.

**Human biomonitoring and its application to mycotoxin exposure assessment: revealing the toxicokinetics of deoxynivalenol in humans**

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Recently, a study by Heyndrickx *et al.* [1] demonstrated the presence of several mycotoxins in urine samples (n=394) of the Belgian population. Deoxynivalenol (DON) and especially its glucuronides were most prevalent, being present in 100% of the urine samples. A risk assessment was performed by deriving estimated dietary intakes from the urinary concentrations. The estimated intake for DON varied
between 0.11-19.57 and 0.03-10.08 µg/kg bw/day for children and adults respectively. This could imply a health risk as 56-69% of children and 16-29% of the adults were estimated to exceed the tolerable daily intake for DON (1 µg/kg bw/day) depending on the approach applied. However, it has to be highlighted that there are still a lot of uncertainties when estimating the DON intake using urinary biomarkers due to the lack of toxicokinetic data of DON in humans. For this reason, an intervention study with 14 adults was designed in order to obtain tentative information about the toxicokinetics of DON in humans. The design was partly based on a theoretical kinetic model developed for DON in humans. Prior to the start of this intervention study, each volunteer had to follow a DON-restricted diet for 2 days. Additionally, each volunteer received a bolus of DON through a naturally contaminated breakfast while remaining on the DON restricted diet for the rest of the day. Urine samples were collected at different time points in the following 24 h. The urine samples were analyzed for the presence of DON as well as its major metabolites de-epoxy-deoxynivalenol, deoxynivalenol-3-glucuronide and -15-glucuronide, using a targeted LC-MS/MS method. As the presence of unknown metabolites can lead to an underestimation of the exposure, additionally an untargeted screening was performed using HR-MS. Toxicokinetic parameters such as the excretion pattern of DON and metabolites throughout a day and absorption and elimination rates will be calculated using the concentration-time curves in urine. Furthermore, this study could give a decisive answer about the use of morning or spot urine compared to 24-hours urine and for the first time natural inter-individual variations will determined within this group of volunteers. The obtained knowledge will serve to develop a standardized method to estimate DON intake by means of biomarker analysis.

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References
Changing trends in the occurrence of ergot mycotoxins in Western Canada

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Ergot contaminated feeds and health problems in both animal and human populations have been known for centuries. In Western Canada, historically, few problems have been encountered. In the past 3-4 years, ergot contamination in feed has increased dramatically. Changing moisture and temperature conditions, no till cultivation and roadside weed control practices have been suggested as contributing factors. The Claviceps fungal species invade the plant during the flowering stage. Various grass species, such as rye, are more susceptible since they have a longer flowering period. In Western Canada, a wet month of June results in ideal conditions for ergot sclerotia development. The contaminated crop will become apparent at the honey dew stage of development. Grass type plants produce a variety of ergot alkaloids which vary with plant species. In Western Canada, ergocristine is the predominant alkaloid present, although there is considerable variation. Contamination may be present in grain, forage or silage. Geographically, major contamination has been reported in Alberta and Saskatchewan, with limited contamination in Manitoba. Annual variation appears to be related to differences in regional rainfall. Prairie Diagnostic Services (www.pdsinc.ca) provides analytical services to detect 6 ergot alkaloids in feed. During 2014 and 2015, hundreds of feed samples which were evaluated contained total ergot alkaloid content exceeding 200 μg/kg. This degree of contamination is considered by many to the maximum safe concentration in livestock feed (total mixed ration). Traditional methods of ergot evaluation including visual counting or weighing of the sclerotia bodies are highly variable as compared to chemical quantification. Since the fungus does not grow uniformly in the crop, it is important to collect a representative sample for analysis. In many instances, this is often difficult. Pelleted livestock rations containing screenings have been the major source of animal disease related to ergot in the past 2 years.

Clinical manifestations of disease vary with the extent of exposure. Exposure at high concentrations may produce neurological manifestations or gangrene, whereas lower amounts of exposure produce agalactia (reduced prolactin production), reduced growth and feed consumption. Abortion which has been described in the literature has not been a common manifestation of disease in Western Canada. Other factors relevant to clinical disease include ambient air temperature, duration of exposure, animal species or breed differences. Horses, sheep and swine are more susceptible than cattle. Lactating animals are greatly impacted. Other concerns associated with ergot-contaminated feeds in Western Canada include food safety, export market loss, tissue residues and withdrawal times in food producing livestock. The economic impact of ergot contaminated feeds affects grain producers, feed companies and livestock producers directly.

Mycotoxins in pet food: the occurrence and significance

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Cereal grains and coproducts as well as vegetables can be utilized as raw ingredients in commercial pet food diets formulated for dogs and cats, thus the potential presence of mycotoxin contamination is a constant pet food chemical safety threat. Normal processing of the raw ingredients into final products does not control or reduce this threat. The major mycotoxins of concern in pet food have historically been aflatoxin and deoxynivalenol, and recalls involving these mycotoxins have occurred. Dogs have been shown to be sensitive to the hepatotoxic effects of aflatoxins and numerous incidents of pet food contamination have resulted in deaths of animals after ingestion of the tainted feed. Worldwide, surveys of pet foods have shown the presence of these and other mycotoxins, including zearalenone, T-2 and HT-2 toxin, ochratoxin A, and fumonisins. As analytical capabilities have continued to improve, additional mycotoxins have been reported in pet foods but a lack of safety studies has made interpretation of the reported levels difficult, especially as the limits of quantitation of mycotoxins have continued to decrease to the low μg/kg ranges. Likewise, regulatory limits specific for pet foods and specific for the wide variety of mycotoxins reported are not developed or promulgated in all countries. A robust HACCP-based program of ingredient evaluation, sampling and testing is the best approach to insure final product safety. This presentation will review the incidences of mycotoxin contamination reported in commercial pet foods as well as the adverse effects of different mycotoxins in animals exposed to the tainted feeds.
Trends in occurrence of masked mycotoxins in food and feed

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Masked mycotoxins are plant metabolites of mycotoxins. While typically having a low intrinsic toxicity, masked mycotoxins might be reactivated during mammalian metabolism. In particular, the previously conjugated polar groups could be cleaved from the molecule in the digestive tract of animals, liberating the native mycotoxins. In the last decade, numerous such metabolites have been identified using advanced liquid chromatography-mass spectrometric techniques. The presentation will highlight novel findings and summarize the current knowledge about the identity of masked mycotoxins. From those approximately 40 characterized substances, only a handful were ever isolated or synthesized. One important benefit of the availability of pure masked mycotoxins is the possibility to perform in vitro and in vivo toxicological tests. Another benefit is the possibility to quantify their concentrations in cereals and cereal based food and feed.

In 2015, over 1000 cereal samples were measured with a LC-MS/MS multi-mycotoxin method developed and maintained at IFA-Tulln [1]. Of the 300+ fungal metabolites included in the method, deoxynivalenol-3-glucoside, HT-2-toxin-3-glucoside, nivalenol-3-glucoside, zearalenone-14-glucoside, zearalenone-14-sulfate and zearalenone-16-glucoside are regarded as masked mycotoxins. The paper will highlight the occurrence of those compounds in various cereals, obtained from 45 countries, in comparison with their native toxins.

References

Expect the unexpected – common mycotoxins in uncommon places

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A few years ago, we presented a talk describing all of the good things about mycotoxins. (That statement in itself will probably draw some scowls and arched eyebrows!). But realistically in the world of mycotoxins, it is very helpful to know what toxins and crop combinations may be possible under certain weather conditions. In fact, many companies try and predetermine what types of problems may be present at harvest time based on the past history. Some feed companies formulate animal diets by using the commodities that will pose the least risk. Many agencies complete pre harvest surveys to confirm initial suspicions so that producers and manufacturers can have a harvest plan in place. When there are so many things that must be managed with mycotoxins, it is good to know that some things are at least a bit constant right?

This constant may be changing, perhaps just a bit, or at least we collectively may need to be more aware of the possibilities of toxins in areas where they weren’t problematic in the past. This presentation, ‘Expect the unexpected – common mycotoxins in uncommon places’ takes a look at the mycotoxins Trilogy Analytical Laboratory is finding in a few unusual places and combinations. Some findings probably represent isolated issues, some findings need more analytical testing over the course of several seasons. Some findings seem rather logical while others, well seem unexpected. In any case, these ‘unexpected places’ provide new challenges for analytical chemists working with mycotoxin methods.
Mycotoxin analysis in your hand

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Mycotoxins contaminations of food and feed have a huge economic impact. Mycotoxins impose a risk to human and animal health. Therefore, maximum limits have been established for many commodities. Legislations and guidelines are implemented and enforced in most parts of the world. Since mycotoxins are natural occurring toxins, they cannot be avoided. As a result, significant amounts of commodities are discarded or used for feed or non-food applications at a lower sales price. Financial losses, however, go far beyond the value of the contaminated commodities and may actually affect the entire food production chain. Animal feed contaminated with mycotoxins may cause production losses in livestock production and mycotoxins may cause significant health costs.

Mycotoxins contaminations of crops are unavoidable but mycotoxins can be managed. Good agricultural and good manufacturing practices will help. Monitoring mycotoxin contaminations by testing is necessary to verify the products will meet international regulations and guidelines. Yet, instead of testing large numbers of end-products, a more pro-active approach would have many benefits. During the entire process from field to food or feed critical steps can be identified to monitor mycotoxins. For this approach a mobile, easy to use tool to make quick, on-site decisions is essential. Lateral flow-based tests are well accepted methods for this. For a quantitative result, a lab environment was still required. R-Biopharm now presents the next generation in rapid, on-site mycotoxin testing. We have developed an app, which allows you to use your smartphone as a lateral flow reader.

The present and future of mycotoxin risk management

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Since 2004, the annual BIOMIN mycotoxin survey provides insight into the risks caused by the main mycotoxins found in agricultural commodities. Up to now, more than 30,000 samples from different countries around the world have been evaluated for the presence of aflatoxins, zearalenone, deoxynivalenol, T-2 toxin, fumonisins and ochratoxin A. The survey summarizes the importance of the co-occurrence of various mycotoxins in the different samples.

Until recently, most of the available analytical methods only covered single classes of mycotoxins, such as aflatoxins, type B trichothecenes or fumonisins. Over the past decade, the sensitivity of liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) has increased by 200-fold and it is quickly becoming the reference for multiple mycotoxin detection. The multi-mycotoxin method Spectrum 380® offered by BIOMIN to its customers allows the detection of more than 380 mycotoxins and metabolites in one sample simultaneously. Spectrum 380® provides a more detailed picture on the mycotoxin situation on the field. The analysis of over 2,000 feed samples by Spectrum 380® shows that co-exposure is the rule and not the exception.

Constant monitoring and continual research on the prevention and mitigation of mycotoxin contamination are therefore necessary. Mycotoxins vary in terms of effects and chemical structures and require different ways of deactivation. For example, aflatoxins can be removed efficiently and safely with binders such as specifically selected bentonite. For other mycotoxins, a different strategy is needed. The most scientifically advanced way to combat mycotoxins uses specific enzymes to convert mycotoxins into non-toxic, environmentally safe metabolites; a process known as biotransformation. This process is irreversible and without any side effects, due to its specificity for one structurally related group of mycotoxins. It is well-known in the scientific community that the binding of Fusarium toxins, such as trichothecenes for example is not effective. Though developing biotransformation products requires dedicated and long-term, intensive research, the enzymatic degradation of mycotoxins is certainly the way forward.

Until 2009, there was no legislation in place recognizing feed additives with mycotoxin counteracting properties in the EU. With Regulation (EC) No. 1831/2003 a new functional group of feed additives for mycotoxin deactivation was enacted. Because of the long-standing focus on mycotoxin research,
BIOMIN was able to provide trials and experiments needed for the successful authorization of Mycofix® Secure (bentonite) for pigs, poultry and ruminants. FUMzyme® and Biomin® BBSH 797 for pigs (EU Regulations No 1060/2013, 1115/2014, and 1016/2013). Meanwhile, the EU legislation for mycotoxin deactivation product is seen as a leading standard and more authorities show interest in establishing a similar feed additive category.

**Mycotoxin analysis of food and feed: old problems and new challenges require innovative solutions**

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Mycotoxins are known for a very long time now and the knowledge on toxicity, occurrence and methods to detect and quantify these undesired compounds has increased over time. Although these fungal metabolites accompany humans since centuries there can be observed some major changes in mycotoxin analysis now. For decades, taking samples and sending them to an analytical service provider has been the main method for determining the presence of mycotoxins. In recent years, rapid test methods have become widely available, offering simplicity and ease-of-use to quickly detect mycotoxins on site. With more options to choose from, finding the right tool has gained importance.

On-site testing vs. analytical service? The first step in finding the right testing solution is to decide whether to conduct the test yourself on-site (e.g. in the field or at the production facility), or send the samples to an analytical service laboratory. The more traditional approach of testing samples for mycotoxins is sending it to an analytical service laboratory for analysis. There usually reference methods like HPLC (high performance liquid chromatography) and LC-MS/MS (liquid chromatography-tandem mass spectrometry) will be applied to produce highly accurate results. Reference methods analyze your sample for multiple toxins in one go. For example, the LC-MS/MS multi-mycotoxin method offered by Romer Labs is capable of analyzing up to 18 toxins at a time. The two most popular rapid methods are strip tests (lateral flow devices, LFDs) and ELISA tests. Strip tests are designed to give results as soon as possible, though they can only process a few samples at a time. They are therefore widely used at reception points of the supply chain of agricultural raw commodities. ELISA (enzyme-linked immunosorbent assay) test kits can test up to 44 samples simultaneously. In general, ELISA is the better option when you have 6 or more samples: the price difference is quickly recuperated due to the need to buy fewer kits and it saves time. However, recent trends show that more and more users prefer LFDs. On the one hand, these tests get more accurate and more sensitive with each new generation and furthermore, more user-friendly approaches have been developed. Many users do not want to deal with organic solvents as they are toxic, the disposal of this waste might be problematic, and undesired if a company is not equipped with a laboratory. Romer Labs has developed the innovative WATEX tests, which allow the analysis for several mycotoxins from a single solvent-free extraction.

**Redefining mycotoxin occurrence and control strategies**

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The large number and structural diversity of mycotoxins has impeded rapid quantification using LC-MS/MS owing to varying toxin extraction efficiencies and interferences from feed and food matrices. We have successfully tackled these challenges by developing an accurate LC-MS/MS (ISO17025 accredited) for the detection of multiple mycotoxins using data normalization by means of isotopically enriched version (isotopologue) of particular toxins. Losses during extraction and matrix suppression/enhancement were normalized using labeled mycotoxins used as surrogates and internal standards. Potential internal standard (IS) replacements such as structural analogs were investigated for cost efficiency purpose. Method extension to a wider array of mycotoxin and use of four isotopologues and three structural analogs were investigated to normalize data acquisition for 10 mycotoxin groups and applied to feed samples.

We conducted a worldwide survey of 9,564 samples of feed ingredients (corn, wheat, barley, soybean) and formulated feeds (silage, total mixed rations, finished feeds, mixed cereals) from three harvest years (2012-2015). It was found that the mycotoxins population followed a Gaussian distribution with
measurable concentrations of mycotoxins detected in 98.6% of the samples (average at peak apex: 6 different mycotoxins per sample) and 56,191 discrete instances of mycotoxin contamination detected. Only 215 samples out of the 9,564 samples analyzed contained no detectable mycotoxins. Per sample, the number of mycotoxins detected ranged from 2 to 20, with around 80% of the samples contaminated with 3 to 11 different mycotoxins. The most prevalent mycotoxin families were fumonisins, representing ~25% of the mycotoxin load, followed by trichothecenes B, representing ~21%. The next most prevalent category was the "Penicillium" toxins, comprising ~11% of mycotoxin load. For the first time, analysis of the distribution of Penicillium toxin as well as potential synergistic compounds, such as fusaric acid, has been made possible. The greater complexity of contamination patterns and toxin concentrations in complete feeds compared with individual ingredients was confirmed. Despite widespread, multi-toxin contamination, the majority of samples complied with current U.S. and EU regulatory thresholds for animal feedstuffs.

This survey clearly demonstrated the feasibility and need for global surveillance of a broad spectrum of mycotoxins in raw ingredients and complex feeds by means of a mass spectrometry approach. Affordable, comprehensive surveillance could improve our understanding of how chronic, low-grade contamination affects animal health and how contamination patterns are influenced by the storing and blending of feed ingredients and by environmental conditions and could help refining predictive models, to improve animal production efficiency, and to safeguard the food chain or apply corrective measures to mitigate the impact of large spectrum contamination.

Correlations in the occurrence of fusariotoxins in different contaminated feed materials

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Feed raw materials show different fusariotoxins occurrence depending on the type of culture, as shown by SCOOP survey in 2003. The aim of this study was to identify correlations in the occurrence of fusariotoxins in different contaminated feed materials. This study used the LABOCEA database composed of chromatography analyses run with LC-MS/MS from 2013 to 2015. Twenty-four fusariotoxins were tested for each sample. In this study, we used samples with at least one trichothecene above LOQ and with a minimum of 30 samples per type of feed material. The final database was composed of 256 samples of wheat, 111 samples of barley, 491 samples of corn silage, 232 samples of corn grain, 88 samples of corn humid, 58 samples of hay and 42 samples of grass silage. The correlation tests were run only between mycotoxins of a same group: DON and other type B trichothecenes, and sum of T-2 and HT-2 (T-2/HT-2) and other type A trichothecenes. Three different tests were performed: first, a logistic regression was run in order to determine the threshold quantity of a mycotoxin to have the presence of another mycotoxin; then, the coefficient of correlation (r) was calculated; and last, if the coefficient of correlation was above 0.85, a simple linear regression was calculated between both mycotoxins.

For grass feed materials (hay and grass silage), we could not measure any significant logistic regression nor correlation. This could be explained by the high variability of contamination as described by Gallo et al. [1]. As a consequence the rest of this study will focus on straw cereals and corn materials. DON is the most occurring trichothecene worldwide. The major derivatives of DON are either formed by fungi (the acetylated derivatives: 3-acetyl deoxynivalenol (3ADON), 15-acetyl deoxynivalenol (15ADON)), or bacteria (the de-epoxide derivatives of DON: DOM-1). The threshold quantity of DON to have 15ADON remains lower than 200 \(\mu g/kg\) for corn materials and barley, whereas it is above 800 \(\mu g/kg\) for wheat, meaning that 15ADON is significantly more frequent on corn and barley compared to wheat. Corn grain has the lowest DON threshold level to have 15ADON (80 \(\mu g/kg\)) and also obtained a high coefficient of correlation (\(r=0.86\)) between DON and 15ADON which permits to obtain a linear regression. Unlike 15ADON, the DON threshold level to have 3ADON is very high for all feed materials: from 400 \(\mu g/kg\) (barley) to more than 4,000 \(\mu g/kg\) (corn silage) and no important correlation could be measured. The very low occurrence of DOM-1 is illustrated by very high DON threshold level (between 1,800 and 8,000 \(\mu g/kg\)) to obtain this metabolite. Even if NIV is produced by different fungi than DON, the DON threshold level to have NIV is lower than 300 \(\mu g/kg\) for all materials. However, the different origins of NIV and DON did not permit to set up a correlation. Regarding type A trichothecenes, the threshold level of T-2/HT-2 to have its metabolite T-2 tetraol was below 60 \(\mu g/kg\) for all feed materials apart for corn humid (150 \(\mu g/kg\)) and no correlation could be established between T-2/HT-2 and T-2 tetraol. Finally, a significant correlation and linear regression could be set up only for corn grain between DON and
This study shows that the occurrence of some mycotoxins are correlated in some raw materials. This could be used to manage mycotoxin risk in feed.

References

In a global prevention strategy of Fusarium mycotoxins, new insights into yeast parietal fractions ability to reduce mycotoxins impact in animal

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Fusarium head blight (FHB) is a major disease of wheat, other small grains and corn plant across the world mainly caused by the fungal plant pathogens Fusarium graminearum and F. culmorum. Following infection of the cereal plant, fungus development leads not only to lowered grain yields, quality loss but also to the accumulation of mycotoxins resulting in contamination of animal feed and human cereal food in toxicologically relevant concentrations. These mycotoxins also called fusariotoxins are produced by different species of the genus Fusarium whose occurrence varies markedly depending on the toxin and the fungi responsible for their synthesis, as well as on temperature, moisture, and presence of crop pests. The toxicity of fusariotoxins also varies strongly depending on the toxin, the level and duration of exposure, the animal species and the co-contamination of several mycotoxins. Fusarium mycotoxins induce both acute and chronic effects. At low dose, mycotoxins impair the intestinal health and the innate and adaptive immune system, increasing animal susceptibility to infectious diseases [1]. Several strategies have been developed to manage mycotoxins in feed, including preventive measures and detoxification procedures. Although prevention of mycotoxin contamination in the field and during storage is the main goal of agricultural and food/feed industries, the absence of mycotoxins in the ration of farm animals cannot be fully assured. The European Commission have recognized the possibility of mycotoxin-detoxifying agents usage, defined as ‘substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action’ as feed additives (Regulation (EC) No 386/2009). The use of these mycotoxin-detoxifying agents may have the greatest application for routine avoidance of this constant exposure to low levels of multiple mycotoxins. Among sequestering agents, yeast cell wall (YCW) have some good capabilities in binding different mycotoxins; the adsorption efficiency of YCW have been shown to be linked to the combination of yeast strains and their manufacturing process. Beyond binding efficiency, YCW by their action on epithelial barrier function of the intestine and on intestinal health reduce negative impact of mycotoxins. Phileo would like to share its comprehensive approach to mycotoxin protection in animal in addition to new perspectives of yeast effect on intestinal permeability and immunity.

References

Jump in, the water’s fine—aqueous extraction for mycotoxin rapid tests

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On-site screening is increasingly important for segregation of commodities based on mycotoxin content. Such screening tests are often performed at sites such as grain elevators during harvest. As such, they need to be rapid, and simple enough to be performed by personnel who are not trained chemists. Historically extraction methods developed in analytical laboratories have relied on the use of organic solvents which may be flammable, toxic or environmentally harmful. More recent work at institutions such as the Agricultural Research Service of the U.S. Department of Agriculture have shown that more environmentally friendly extraction with water-based media can replace organic solvents for mycotoxin extraction prior to analysis.

VICAM has developed a proprietary water based extraction solvent that can be used to extract multiple mycotoxins from a single sample. Portions of the extract can then be analyzed for multiple mycotoxins saving time and minimizing impact on the environment as well as eliminating costs of disposing of hazardous organic solvents. Combination of this AQUA™ multi-mycotoxin extraction solution with AFLA-
and DON-® Vertu® quantitative strip tests provides a limits of detection as low as 2.6 ppb for total aflatoxins and 0.2 ppm for deoxynivalenol. Time to result from ground grain sample is less than 10 min.

Sample preparation for LC-MS/MS analysis of mycotoxins

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The use of mass spectrometry methods for multi-mycotoxin analyses provides high levels of detection selectivity and sensitivity. It allows for simultaneous detection of contaminants and the confirmation of their identity. However, the analysis of mycotoxins by LC-MS suffers from the disadvantages characteristic for mass spectrometry detection: quantitation can be adversely affected by the co-extracted matrix. Therefore, LC-MS methods require either the use of internal standards, which can raise the cost of analysis, or the use of matrix-matched standards, which could be difficult as mycotoxins can be naturally present in various crops at non-zero levels. Sample preparation procedures previously used with UV and FL detection can potentially provide an acceptable cleanup for LC-MS detection. Many such methods focused on one mycotoxin compound or one class of compounds, but can still be useful when LC-MS analysis is performed. In this work, we compared the matrix effects for LC-MS/MS detection from extracted samples that did not utilize SPE cleanup to those from samples where SPE cleanup was applied. Similarly to immunoaffinity sorbents, this family of SPE cartridges is recommended for specific mycotoxin classes. We have successfully applied the SPE cleanup for very difficult samples, such as hops, with the use of matrix-matched standards. The results from this preparation method were encouraging with good accuracy and method reproducibility.

Apart from regulated mycotoxins, other compounds in this class are of interest to researchers. One such compound is citrinin. In recent years, laboratories developed a strong interest in the analysis of dietary supplements for mycotoxins. Citrinin has similar properties to the other components of dietary supplements and proved challenging to separate out for accurate testing. Most of the existing sample preparation methods are not optimal for reliable quantitation of this compound. We will share our experience in investigating a method for improved sample preparation for LC-MS analysis of citrinin.

Overcoming matrix interference in the detection of mycotoxins in foods

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Aflatoxin B1 is a well-known potent human carcinogen produced by toxigenic fungi. Given its ubiquitous presence in a wide variety of foods and beverages, aflatoxin B1 levels must be measured and monitored to prevent contaminated food from reaching the consumer. Enzyme-linked immunosorbent assays (ELISAs) are frequently employed as a rapid and inexpensive method to screen samples that may contain aflatoxin B1 concentrations above the legal permissible limit. To date, many available ELISAs are limited in the range of commodities that can be tested because they are subject to matrix interferences and require an additional cleanup step. The aim of this study was to evaluate if a single ELISA kit can accurately detect aflatoxin B1 in commodities that typically pose matrix interferences, including nuts, spices, and other common cooking ingredients. The various comestibles were purchased from local markets and evaluated for matrix interferences. A Student’s t-test was performed to determine any significant differences between the extracted commodity and extraction solvent alone. Samples were then fortified with aflatoxin B1 at various concentrations ranging from 2.5-20 μg/kg and measured by a previously developed competitive inhibition ELISA. The % recovery and %CVs were calculated. All food items exhibited minimal matrix interferences. The mean signal of the commodities did not demonstrate a statistically significant differences compared to the mean signal of the extraction solvent alone (p<0.05). Most commodities showed excellent recoveries of 82-111% with %CVs of less than 13%. The data demonstrates that a single ELISA kit can be used to successfully quantify aflatoxin B1 in most commodities without the need for special extraction methods or cleanup procedures.
Best practices for rapid mycotoxin testing

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The Food Safety Modernization Act (FSMA) is increasing attention to companies’ food safety risk management and internal quality procedures. At the same time, the industry struggles with repeated seasons where mycotoxins are prevalent in wheat or corn. Proper sampling is the most important step to achieving accurate results with a reliable, rapid test. Representative sampling, combined with EnviroLogix’ support and improved QuickScan Flex technology, will achieve reliable, traceable, mycotoxin results in less than 10 min.

The use of biomarkers to assess efficacy of mycotoxin eliminators in vivo

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As our weapons in the fight against mycotoxins become more sophisticated, so must the methods we use to test their efficacy. Recently, Impextraco developed a wide range of in vivo protocols to determine the effect of mycotoxins in an early stage, using biomarkers. One of the most recent techniques, the one using biomarkers, has been introduced a few years ago in all our in vivo trials. Biomarkers are characteristics that are objectively measured and evaluated as indicators of biological processes, pathogenic or pharmacological responses to an intervention, such as mycotoxin ingestion. They need to be reliable, fast and easy to measure in order to find significant effects as early as possible in the animals’ reaction to mycotoxicosis. To find mycotoxin induced changes in the animals’ metabolism, Impextraco implements different strategies at the same time: blood biochemistry, flow cytometry and histopathology are just a few amongst them.

All mycotoxins affect animal health status due to their toxic characteristics, but they all work in a different way. Each mycotoxin needs a specific blood parameter to investigate its effect on the animal. Aflatoxin for example is hepatotoxic and blood parameters indicating liver functionality can reveal information to further increase our knowledge on the best way to eliminate its toxicity. Ochratoxins on the other hand are nephrotoxic and need different biomarkers to investigate their effects on kidney impairment due to ochratoxicosis. To gain information on the effect of mycotoxins on the animals’ immune reaction, flow cytometry is used in our in vivo research as a tool. Flow cytometry is a impedance-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in basic research, clinical practices and clinical trials. In our research it is used to quantify the number of circulating immune cells, namely lymphocytes. This information enables us to determine the effect of different mycotoxins on the immune response in the animal and the efficacy of Elitox® in supporting the animal immune reaction when encountering mycotoxicosis.

Neogen launches the Reveal Q+ MAX system (mycotoxin aqueous extraction)

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In its 34-year history, Neogen Corporation has been a firsthand witness and contributor to the ever evolving field of rapid diagnostics for the detection of mycotoxin residues. In that time we have seen technology evolve to provide faster, more inexpensive, accurate tools for this purpose while at the same time making them easier and easier to use granting a full skill spectrum of individuals access from the highly trained to the novice. Never before have individuals had so many technology choices for mycotoxin control. With this proliferation of technology, the user has become more sophisticated in their mycotoxin management needs. No longer are test kits alone sufficient for comprehensive mycotoxin management. Many users require conformational services, pure standards, naturally incurred reference materials, proficiency programs, data management with documentation, and predictive modeling. This comprehensive package has become an expectation in today’s complex regulatory environment.
Neogen Corporation would like to share our comprehensive approach to mycotoxin management in addition to our recently launched Reveal Q+ MAX system. Neogen Corporation has developed the Reveal Q+ MAX (mycotoxin aqueous extraction) method for the detection of the 6 commonly analyzed mycotoxins with T-2 / HT-2 and ochratoxin in final stages of development. Neogen a leading supplier and developer of ELISA test kits, immunoaffinity columns, and lateral flow assays has now enhanced the very robust quantitative lateral flow method Reveal Q+ by developing a water based universal extraction that not only can be easily disposed but allow the user to test for multiple toxins from the same extract greatly reducing sample prep time. The Reveal Q+ MAX platform is a rapid lateral flow device used to quantitate levels of mycotoxins in grains and foodstuffs. These are very rapid ranging in runtime from 3-6 min and show exceptional precision and accuracy when compared to HPLC methodology. Results are fully quantitative when analyzed using any of the Neogen Accuscan family of lateral flow readers and can be uploaded and managed through a software suite or used as a stand-alone device.

Mycotoxin risk management in the grain supply chain

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Eurofins is a leading international group of laboratories providing an unparalleled range of testing and support services for pharmaceutical, food, environmental and consumer products industries as well as for governments. The Eurofins Group has built up a global network of state-of-the-art food testing laboratories and competence centres that perform more than 80 million assays per year to establish the safety, composition, authenticity, origin, traceability and purity of food. Mycotoxin and plant toxin contamination could have a major impact on the international trade of agricultural products. Depending on its origin, grain might partially contain Fusarium toxins, aflatoxins and/or ochratoxin A, but also emerging toxins as Alternaria toxins or pyrrolizidine and tropane alkaloids. To ensure the safety of grain and grain products and their compliance with EU and Codex legislation, traders are required to quantify the mycotoxin levels within their lots. Due to the extremely inhomogeneous distribution of some toxins, there is often the risk of considerably misinterpreting a good’s quality. Very often, a time and cost effective strategy within the supply chain is needed to achieve the highest quality along with lowest rejection rates. Currently, Eurofins has set up a global sampling and inspection service in 32 countries for the active surveillance and control of the entire food supply chain from farm to fork. Consequently, these programs minimize the risks by:

- consults to avoid the risks before they arise;
- consults to set up sufficient monitoring and sampling plans;
- representative sampling at all points of the supply chain, on fields, at grain-elevators, at silo stations, at mills, at retailers;
- offering reliable and fast on-site testing by means of the rapidust® system for major toxins;
- comprehensive check-ups by state-of-the-art multi-toxin screening methods;
- single and multi-toxin reference methods in an accredited lab;
- comprehensive knowledge of European and international food regulations; and
- audits and food safety inspections.

Training and educating the different industry parts from agriculture over producers up to the final distributors always make part of the program provided by the analytical experts of Eurofins.

B ZERO®, master-curve calibrated immunoassays for cost-effective detection of mycotoxins

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Thanks to their reliability, sensitivity and precision, ELISAs are widespread tools for the detection of a number of contaminants in food and feedstuffs. The analysis is based on the reaction between a binder (typically, an antibody) and one or more ligands (the contaminant or secondary antibodies). Such binding reaction is affected by environmental conditions (i.e., light, temperature and incubation time) as well as degradation of binders and/or tracers; thus, the analyst has generally to run a calibration curve within each analytical session, even when analyzing one or few samples. The aim of this presentation is to show the characteristics of a new generation of quantitative microplate enzyme immunoassays for the detection of chemical contaminants. The main feature of these new tests is the avoidance of traditional
calibration, running just the ‘zero’ standard. Proper calibration curves, which B/Bo values are optimized for each kits batch, are established (‘master curves’). Once the ratio between each sample absorbance (B) and the ‘zero’ standard absorbance (Bo) is obtained, the B/Bo value of each sample is interpolated onto the master curve to get the quantification of the analyte. This master-curve ELISA tests are called B ZERO®. This new technology allows the cost-optimization of the analysis as for each analytical session just one microwell is used for the standards, while all the others can be used for samples. This is much significant for users that have few samples to test: considering a 96 plate, if during each analytical session just 3 samples are tested, with the ZERO® kit it would be possible to test two times more the samples that could be analyzed with a traditional ELISA kit. The possibility of avoiding calibration presents further advantages: fewer reagents have to be used, the assay performance is less time consuming and the handling error probability is reduced. Verification of the ZERO® kits performances was carried out by comparison of concentration values with data obtained with the traditional kit, testing the same sample extracts in parallel. Results showed that both accuracy and precision of the B ZERO® AFLA and B ZERO® DON were very close to those of Celer® AFLA and Celer DON®, the corresponding kits in which the calibration was performed in each run.

As a conclusion, thanks to the stability of the reagents and to the assays robustness, the B ZERO® kits showed similar performances as the relative parental kits. Using the manufacturer master-curve makes the B ZERO® kits the most suitable option for those testing a few samples per session: a higher number of wells available for sample analysis on the microtiter plate makes the cost of each test much lower.

**Reliable biochip array technology for the fast, sensitive and semi-quantitative detection of multiple mycotoxins in animal feed**

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Certain fungi produce mycotoxins as secondary metabolites, which are harmful to humans, domestic animals and livestock. Mycotoxins are found in a wide range of food and feeds, particularly in areas with climates of high temperature and humidity. Aflatoxins, fumonisins, ochratoxins, trichothecenes A (T-2 toxin, HT-2 toxin), trichothecenes B (deoxynivalenol), and zearalenones are the main known groups of mycotoxins. In Europe, maximum permitted limits for aflatoxin B1 are set and guidance values for the other mycotoxins are recommended. The determination of other mycotoxins, such as diacetoxyscirpenol, ergot alkaloids and paxilline, have gained importance for food and feed safety. Biochip Array Technology (BAT) allows the simultaneous determination of multiple analytes from a single sample, which consolidates testing and increases the screening capacity. This leads to the reduction of the number of samples to be assessed by confirmatory analysis. This study reports the applicability of this technology to the simultaneous semi-quantitative determination of a broad range of mycotoxins to accommodate the lowest guidance limits established for a particular feed type.

Two different biochip arrays were developed to cover the detection of the most prevalent mycotoxins (Myc 7) and to expand the detection capability even further, the addition of diacetoxyscirpenol, ergot alkaloids and paxilline (Myc 10). Simultaneous competitive chemiluminescence immunoassays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator analyzer, were employed for both biochip arrays. Mycotoxins were extracted from feed by a single generic liquid/liquid extraction. Myco 7 biochip array was validated based on 2002/657/EC for detection of aflatoxins, ochratoxin A, fumonisins, trichothecenes A (T-2 toxin, HT-2 toxin), trichothecenes B (deoxynivalenol) and zearalenone including metabolites. The screening decision levels ranged from 0.25 μg/kg (ochratoxin A and aflatoxin B1 assays) to 100 μg/kg (deoxynivalenol assay) for sensitive detection, and from 3.12 μg/kg (ochratoxin A and aflatoxin B1 assays) to 1,250 μg/kg (deoxynivalenol assay) for monitoring level. The overall intra-assay precision, evaluated by repeatability and within-laboratory reproducibility studies for all the assays on Myco 7 biochip expressed as CV (%) at different fortification levels were <11% and <12%, respectively. Analysis of animal feed samples from FAPAS proficiency testing programme and FAPAS QC showed that all values obtained with Myco 7 biochip arrays fell within the range assigned by FAPAS for samples presenting single or multi-mycotoxin contamination. In conclusion, BAT allows the reliable simultaneous, semi-quantitative screening of a broad range of mycotoxins at levels to accommodate the lowest established global guidance limits.
Smart reader technology and data management for mycotoxin analysis

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Mycotoxin analyses of feed and grain has transitioned from the central laboratory to the point-of-receipt due to advancements with lateral flow devices and water-based extractions. Smart reader functionality allows non-laboratory personnel to perform testing with improved data handling for traceability, simplified interpretation of test results, and reduced operator error. Charm Sciences developed the Charm EZ®-M system with ethernet capability to allow data transmission directly to a computer or LIMS (Lab Information System). The Charm EZ-M system detects lab errors, such as under dispensing and insertion of previously run strips, and tracks the errors on the built-in SD card. Each test strip is color coded so the system sensor can identify the correct mycotoxin and upload the test kit parameters, including calibration curve and incubation specifications. The built-in incubator on the Charm EZ-M system automatically interprets the test result at completion of the incubation so no further operator manipulation is needed.
TUESDAY, JUNE 7, 2016 – Parallel session 5

MyToolBox: Safe Food and Feed through an Integrated ToolBox for Mycotoxin Management

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With the kick-off meeting of the EU project MyToolBox early March 2016, an integrated approach to reduce molds and mycotoxins along the whole food and feed chain has officially been launched. The 4-year, €5 million project will develop a series of integrated measures, which will enable a significant reduction in losses of crops due to fungal and mycotoxin contamination.

The occurrence of fungal and subsequently mycotoxin contamination in various crops is of major concern since it has significant implications for food and feed safety, food security and international trade. MyToolBox mobilizes a comprehensive multi-actor towards reducing risk due to mycotoxin contamination in crops all along the feed and food chains, involving pre-harvest interventions undertaken at farm level, together with post-harvest interventions undertaken from storage through to food processing into the finished food or feed product. The project consortium includes some 40% partners from industry of which 5 partners are end users from the farming community, agronomists and professionals working in agriculture and food manufacturing. The MyToolBox project will not only pursue a field-to-fork approach along the food and feed chain, but will also consider safe use options of mycotoxin contaminated batches such as microbial energy conversion to efficiently produce biofuels. The consideration of the entire chain to ensure food & feed security and safety within a sustainable economic environment, is a major motivation behind MyToolBox. The mycotoxin commodity combinations that will be addressed are the most prevalent Fusarium mycotoxins (deoxynivalenol, T-2/HT-2 toxins, zearalenone and fumonisins) in wheat, oats, corn and animal feed chains, ochratoxin A in wheat and aflatoxins in corn, peanuts and dried fruit (figs).

Depending on the commodity and the type of intervention, the MyToolBox consortium aims to achieve mycotoxin reductions of 20-90%. Moreover, information and decision support tools will be developed for each level of the chain and will be integrated into the ergonomic and secure web-based MyToolBox platform that will also be accessible over all mobile platforms. As such, the MyToolBox platform will guide the end user to the most effective measure(s) to reduce biological contamination in crops, and will provide the necessary intelligence to ensure these measures take into account the prevailing conditions such as geographical location, meteorological conditions, land-use, crop management, storage and intended end use with relevance to specific crops. In cooperation with 3 partners from China, MyToolBox will also aim to develop a sound scientific basis for standard settings in China for authorization of mycotoxin detoxifying feed additives, and consequently improve market access of relevant EU products.

From MycoRed to MycoKey: integrated and innovative key actions for mycotoxin management in the food and feed chain

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MycoKey is the new project funded by European Commission under the Horizon 2020 program, Societal challenge 2 ‘Food security, sustainable agriculture and forestry, marine, maritime and inland water research and the bioeconomy challenge’ – topic ‘Biological contamination of crops and the food chain’.

In the past, several actions have been developed to counteract mycotoxins effects and risks in the single stages of the value chain, often difficult to be applied by common farmers and SMEs. The previous FP7 MycoRed research collaborative project, coordinated by CNR with a large consortium of 25 partners (2009-2013), developed solution driven methodologies. Handling procedures and practically useful tools were implemented and improved to reduce significantly both pre- and post-harvest toxin contamination of selected and economically important commodities in food and feed chains. MycoKey is built on the MycoRed basis and aims to generate innovative and integrated solutions that will support stakeholders in effective and sustainable mycotoxin management along food and feed chains. The project will contribute to reduce mycotoxin contamination mainly in Europe and China, where frequent and severe mycotoxin contaminations occur in crops, and where international trade of commodities and...
contaminated batches are increasing. MycoKey will address the major affected crops corn, wheat and barley, their associated toxigenic fungi and related mycotoxins (aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, fumonisins). The project will integrate key information and practical solutions for mycotoxin management into a smart ICT tool (MycoKey app), providing answers to stakeholders, who require rapid, customized forecasting, descriptive information on contamination risk/levels, decision support and practical economically-sound suggestions for intervention. Tools and methodologies will be strategically targeted for cost-effective application in the field and during storage, processing and transportation. Alternative and safe ways to use contaminated batches will be also delivered. The focus of Mycokey will be: (1) innovating communications of mycotoxin management by applying ICT, providing input for legislation, enhancing knowledge and networks; (2) selecting and improving a range of tools for mycotoxin monitoring; and (3) assessing the use of reliable solutions, sustainable compounds/green technologies in prevention, intervention and remediation. The multi-disciplinary consortium, composed by scientific, industrial and association partners (32), includes 11 Chinese institutions and will conduct the 4 years program in a framework of international networks.

The mycotoxin metrology capacity building and knowledge transfer (CBKT) project

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The CBKT project addresses the needs expressed to target the improvement of metrological capabilities in the developing world, and responds to the metrology needs in the developing world for food and feed analysis. The issue of food safety and trade is a major concern for countries developing metrology and quality assurance systems. Ensuring the safety of food has been a major focus of international and national action over the last years. Both microbiological and chemical hazards are of concern, including the contamination of food and feed by mycotoxins (toxic metabolites of fungi), which are significant sources of food-borne illnesses. The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish regulations on mycotoxins in food and feed in the last decades to safeguard the health of humans, as well as the economic interests of producers and traders. Over 100 countries have implemented specific regulatory limits for mycotoxins in foodstuffs and feedstuffs, and these need to be supported by a sound measurement infrastructure for mycotoxin analysis in order to enforce and verify products, protect populations and avoid technical barriers to trade in food stuffs. The CBKT project is designed to allow the International Bureau of Weights and Measures (BIPM) and National Metrology Institutes (NMIs) to work together to strengthen mycotoxin metrology infrastructure; provide knowledge transfer to scientists developing capabilities in this area; and enable NMIs to characterize selected pure mycotoxin materials, provide mycotoxin calibrants and matrix reference material and proficiency test materials to support mycotoxin testing laboratories within their countries.

Speed presentations

Short presentations by selected poster presenters to provide an overview of their research and inspire the audience to visit their poster.

P1
The epidemiology of Fusarium langsethiae in oats
Heidi U. Aamot
Norwegian Institute for Bioeconomy Research (NIBIO), Norway

P28
Transfer of aflatoxin B1 from feed to lambari fish (Astyanax altiparanae)
Andrezza M. Fernandes
Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo, Brazil

P39
Aflatoxin exposure, gut function and child growth in Nepal
Sinead Watson
Queen's University Belfast, UK
P57
Evaluating the BGY fluorescence signal in corn kernels inoculated with various aflatoxin producing fungi using fluorescence hyperspectral imaging
Zuzana Hruska
Geosystems Research Institute, Mississippi State University, USA

P60
Design of a novel microfluidics platform for mycotoxin food contaminant determination
Jonathan Loftus
School of Biotechnology, Dublin City University, Ireland

P89
Use of soil bacteria in biological control against aflatoxins: the AFLAFREE project
Florence Mathieu
Laboratoire de Génie Chimique, UMR 5503 (CNRS/INPT/UPS), France

P96
The impact of climate change on free and masked fumonisins
Chiara Dall'Asta
Department of Food Science, University of Parma, Italy

P98
rtfA, a putative RNA-pol II transcription elongation factor gene, is necessary for normal morphological and chemical development, proper response to oxidative stress and pathogenicity in Aspergillus flavus
Jessica M. Lohmar
Department of Biological Sciences, Northern Illinois University, USA

P103
Relationship between ochratoxin A accumulation and pathogenicity of Aspergillus spp. in grapes
Edward Sionov
Institute for Postharvest and Food Sciences, Agricultural Research Organization, The Volcani Center, Israel

P127
Using enzymes and microorganisms to modify the mycotoxin deoxynivalenol
Nina Wilson
Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, USA
The importance of sampling in food and feed safety assessment

Claudia Paoletti¹ and K.H. Esbensen²
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The general principles for safety and nutritional evaluation of foods and feed and potential health risks associated with hazardous compounds have been developed by FAO and WHO [1]. Nevertheless, the crucial role that sampling has in foods/feed safety assessment has never been explicitly recognized. High quality sampling should always be applied to ensure the use of adequate and representative samples as test materials for all the steps of food/feed safety assessment: hazard identification, toxicological and nutritional characterization of identified hazards, as well as estimation of quantitative and reliable exposure levels of foods/feed or related compounds of concern for humans and animals [2]. The different types of substances under study which are present in food/feed matrices and commodities, raw or semi-processed, pose both general and specific challenges to the development of appropriate sampling strategies and analytical detection methods. Although it is well recognized that both sampling and analytical errors affect the reliability of any final risk estimation, traditionally much more attention has been devoted to the development and improvement of analytical methods, as compared to the development of appropriate sampling plans. But the reality is that analytical results are of low or no value, no matter the quality of the method used, if the sampling process is not representative of the entire field-to- aliquot pathway.

The Theory of Sampling (TOS) has developed over the last six decades a complete theory of heterogeneity, sampling procedures and sampling equipment assessment, the importance of which was first recognized in the mining and geological sectors, but later on successfully applied in other areas, including food and feed safety assessment. Over the course of the last 10-15 years the universality of TOS principles has been proven thoroughly, demonstrating that all sampling processes, irrespective of the nature of their target lots, need to be structurally correct (unbiased) in order to ensure a sufficient degree of accuracy and precision [3]. This is true also when assessing foods and feed safety, including food/feed contaminants, additives, naturally occurring toxins/ anti-nutrients, or contaminating microorganisms, and whole foods/feed derived from genetically modified plants/animals. More specifically, TOS allows estimating the variability remaining after all sources of sampling bias have been removed, i.e., the variability intrinsic to the specific material under investigation. From a food and feed safety perspective, this constitutes the level of unavoidable risk associated with any given survey. No other sampling framework allows objective quantification of the risk as a direct function of the specific heterogeneity properties of the test material. On the contrary: all other sampling frameworks rely on specific distributional assumptions, do not characterize heterogeneity patterns stemming from the specific properties of the test material, and do not include an estimation of the risk associated with sampling surveys [4]. For these reasons we consider that TOS provides a complete framework to ensure accuracy and precision of all sampling steps involved in any given scenario, starting from the primary sampling all the way to the subsequent secondary sampling steps involved in the field-to-fork continuum necessary to monitor foods and feed safety. Therefore we propose to explicitly recognize the central role of sampling in foods and feed safety assessment and to integrate TOS in the well-established FAO/WHO risk assessment approach in order to guarantee a transparent and correct frame for the safety assessment of foods and feed and the many steps of the subsequent decision making process. A key example of successful implementation of this approach regarding GMO detection and quantification was published recently [5-7].

References
Development of an online FAO mycotoxin sampling tool and its application to aflatoxin sampling protocol for corn and groundnuts in Africa* 

*presented by Sheryl Tittlemier, Canadian Grain Commission, Canada, on behalf of A.B. Slate and T.B. Whitaker, Biological and Agricultural Engineering Department, North Carolina State University, USA; slate@eos.ncsu.edu

Agricultural commodities are often inspected for mycotoxins and classified into acceptable and unacceptable categories depending on whether the mycotoxin level in a sample(s) taken from the lot is below or above a predefined accept/reject limit, respectively. Because of the large variability associated with the sampling, sample preparation, and analytical steps of a mycotoxin test procedure, some lots will be misclassified by the mycotoxin sampling plan. It is important to be able to predict the level of each risk associated with a sampling plan design so that sampling plans can be designed to reduce one or both risks to acceptable levels for available resources. The variability and distribution among sample test results have been studied for approximately 24 different mycotoxin/commodity combinations. Using the variability and distribution information among sample test results, a method was developed to calculate operating characteristic (OC) curves which provides a measure of the buyer’s and seller’s risks of a specific mycotoxin sampling plan. An OC curve calculator, using EXCEL and Visual Basic, was developed by the authors for general use by regulatory agencies, commodity organizations, food and feed manufacturers, and international health organizations. The FAO has developed an enhanced web-based form of the OC curve calculator. The OC calculator, currently called the FAO Mycotoxin Sampling Tool can be found on the web under FAO Food Safety Risk Analysis Tools (http://www.fstools.org). The FAO Mycotoxin Sampling Tool can compare the performance of up to 10 different mycotoxin sampling plan designs at one time. By inputting the sampling plan design elements (sample size, test portion size, number of aliquots quantified, and accept/reject limit) into the FAO Mycotoxin Sampling Tool, the program will calculate the chances of accepting and rejecting lots at all lot concentrations (OC curve) for sampling plan designs of interest. At the request of several East African organizations (IITA, COMESA, and AATF), the FAO Mycotoxin Sampling Tool was used to design two aflatoxin sampling plans, one for corn and one for groundnuts. The corn sampling plans recommends a 5 kg laboratory sample and a 10 µg/kg accept/reject limit. The groundnut sampling plan recommends a 20 kg laboratory sample and a 15 µg/kg accept/reject limit. The two aflatoxin sampling plans along with their risk evaluation have been recommended to African leaders for their consideration.

Sampling of grain for mycotoxins: understanding the factors that control sampling precision 

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Sampling of grain for mycotoxins is challenging in that some mycotoxins, such as OTA, have very low permitted levels and the kernels carrying the mycotoxin may be highly localized within the bulk of the grain as 'hot spots'. Conventional means of sampling under these circumstances can be shown to lead to a high variance in the sampling result. Unbiased sampling of granular materials demands that a few simple criteria be met in the sampling system design and when these are fulfilled, modern statistical theory of sampling can be applied to determine not just the sampling variance but the entire sampling distribution for a particular methodology, if the mode of occurrence of the target analyte in the lot to be sampled is known or can be reasonably covered by a suite of occurrence models. The paper discusses current methods of grain sampling and the analytical methods that have been applied with the objective of quantifying sampling precision. Statistical results for a number of sampling scenarios using more general statistical techniques are then presented. These analyses pinpoint the vulnerabilities in the conventional methods. A new mechanical sampler design particularly suited to sampling large flows of grain is presented and it is shown how the sampler can virtually eliminate the sampling uncertainties that arise especially when sampling for mycotoxins.
Sampling of cereals: development of a protocol for mycotoxins analysis

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In 2006, the European commission set limits for some mycotoxin contents for food and feed consumption and published some directives to give the methodology to make the sampling of the batches in such a way to get a representative sample. The operators found these protocols strict, not practical and time consuming. But in the same time they knew that the composition of batches of cereals is rarely homogeneous and, in particular, the distribution of contaminants is not uniform. To determine if it was possible to simplify the official protocols, ARVALIS and its partners have run studies with the following aims: (1) to collect data about the distribution of mycotoxin in a silo; (2) to compare two alternative protocols with the existing protocol; (3) to determine the relationship between the number of increments that constitute the representative sample and the total analysis uncertainty; and (4) to test if it is possible to reduce the weight of the laboratory sample defined by the directive.

12 silos have been selected with wheat or corn to evaluate the DON and fumonisins distributions and 100 increments were extracted according a grid. The mycotoxin contents were very diverse and the higher the mycotoxin average content was, the greater the variability was. The comparison of two alternative protocols (routine and normative) to the directive protocol was run in many situations: flowing cereals, static batches with automatic or manual system of extraction. So, 22 silos were tested, with a large range of DON contents including the lawful limits. The results showed that the two alternative protocols gave the same evaluation of the mean DON level allowing for analytical uncertainty. One experiment based on 16 silos with DON allowed the calculation of the uncertainty of the estimation of the mycotoxin content of a batch according to the number of increments. From a relatively small number of increments, the gain of accuracy caused by an additional sample became negligible. It means that the operators can reduce the resources devoted to the sampling and reduce the cost. Finally, 4 samples were reduced using a conical divider and 5 weight levels were tested. The results have shown that it is possible to reduce the mass of the laboratory sample from 10 to 3 kg for the detection of Fusarium mycotoxins. The outcomes of these studies have been validated by the International Standardization Organization (ISO). In 2014, the revised directive allows the normative protocol for batches over 500 t. To meet the changes in regulations, in 2015, we conducted a study on sampling of ergot based on 4 wheat batches and we confirm that it is possible to use a simplified protocol with an acceptable number of increments to get a representative sample.

Sources of variability in measuring aflatoxin and the role of sampling

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The One-Sample-Strategy (OSS) in Texas and the Aflatoxin Proficiency Testing and Control (APTECA) program in Africa document how implementing a quality system can reduce aflatoxin measurement variability. The quantification of variability in aflatoxin measurement is important to food safety risk management. The sources of variability during aflatoxin measurement include sampling, sample preparation, and analysis, which may be further partitioned to include sample particle size reduction, subsample size, analyst, testing platform, reagents, scale, sample matrix, detection, and instrument calibration. Historically, several studies indicate random error associated with sampling may range from 23% to 82%, reported as the coefficient of variation (CV) and evaluated in a single laboratory. Sample particle size reduction and the size of the subsample determine the number of particles from which the aflatoxin is measured to retain the representative properties of the sample. Recently, one laboratory community participating in APTECA program experienced a 78% CV during their first two rounds of proficiency testing, documenting that between laboratory variability is another major error source for aflatoxin measurement in the market place. Upon completion of a one day training exercise under the APTECA program, this laboratory community reduced the composite proficiency test CV to 32% and 16% using two APTECA approved aflatoxin test kits. Both OSS and APTECA programs incorporate third-party verification of analytical results by Texas A&M ISO 17025 laboratories in College Station, TX and Nairobi, Kenya, respectively. Millers adopting the APTECA program experienced a four-fold reduction in laboratory random error characterized by performance curves. To summarize the contribution of sampling, test kit, and analyst variability, two uncertainty estimates were calculated for worst and best case scenarios. The total uncertainty yielded a worse-case scenario of 198% and the best-case scenario uncertainty estimate of 64% variability using a coverage factor of two. The studies
included in this presentation reveal the importance of a quality system to manage aflatoxin measurement variability in the market place.

Sampling for mycotoxins in feed – correct heterogeneity characterization

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The presence of mycotoxins, in particular aflatoxin B1, can cause significant health problems as well as severe economic loss, and is therefore regulated with respect to maximum acceptable concentration for various feed- and foodstuffs. International regulatory authorities have recognized the importance of representative sampling, and sampling guidelines have been formulated which only partly comply with the Theory of Sampling (TOS). Especially heterogeneity characterization methods, which are a necessary requirement to develop valid sampling protocols or validation assessments of existing sampling operations, are currently not mentioned in existing guidelines. The present paper presents structural guidelines for correctly designing experimental heterogeneity characterizations, allowing evaluation of sampling representativeness and determination of optimal number of increments per composite sample. A practical example is given on how to extract information from variographic analysis, based on an extensive field trial performed to determine aflatoxin levels in a feed mixture. Full results and practical guidance regarding mycotoxins sampling, including correct design and operation of sampling devices as well as further explanations on how to develop sufficient sampling protocols can be found in Wagner, 2015.

Development of aflatoxin sampling plans for Brazil nuts

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Brazil nuts are the seeds of Bertholletia excelsa Humb. & Bompl., trees that grow wild in the Amazon rainforest. Because of climatic conditions and characteristics of the extractivism activity associated with a complex collection and transportation logistic, toxigenic fungi and aflatoxin (AF) production are more likely to occur with Brazil nuts compared to other types of tree nuts (1,2). Aspergillus nomius is a major producer [3,4] of AF in Brazil nuts, with 100% of isolates producing AFs. In 2003, the EU issued Directives on Brazil nuts with huge implications on Brazil’s exports that has switched to new export markets and increased commercialization in the domestic market [4]. Aflatoxin sampling plans for both shelled (SBN) and in-shell Brazil nuts (ISBN), and AF risk components in ISBN nuts studies were developed in Brazil [5]. The studies were based on unbalanced nested design. The sampling of both SBN and ISBN of 13 lots of ISBN and 25 lots of ready to market were carried out in the State of Para, in the Amazonia Region. Aggregate samples of 200 kg and 400 kg were taken from lots of SBN and ISBN, respectively. SBN and ISBN aggregate samples were subdivided in 20 laboratory samples (called samples) of 10 kg and 20 kg, respectively. Each ISBN sample were shelled and sorted in to five fractions (risk categories): good kernels, rotten kernels, good shells with kernel residue, good shells without kernel residue, and rotten shells, and analyzed for aflatoxins. The kernel:shell ratio mass (w/w) was 50.2/49.8%. The fractions were analyzed separately, and a mass balance equation was used to calculate the total contamination in the 20 kg sample of ISBN. Sampling (10 kg), sample preparation (50 g test portion) and analytical variances (1 aliquot) for the SBN aflatoxin test procedure was calculated with the sampling step contributing about 98% of the total variance at 10 µg/kg. AF results in both SBN and ISBN were shown to fit the negative binomial distribution. Operating characteristics curves were developed for sampling plans that provides information about the consumer’s and producer’s risk facing the proposed limits in ready-to-eat SBN (10 µg/kg) and for further processing SBN (15 µg/kg) brazil nuts by Codex Alimentarius. Codex sampling plans were then established for ready-to-eat and for further processing SBN. The risk component [5,6] analysis provided evidenced that Brazil nut shells were contaminated with aflatoxin. Rotten nuts were found to be a high-risk fraction for aflatoxin in ISBN lots. Rotten nuts contributed only 4.2% of the sample mass (kg), but contributed 76.6% of the total aflatoxin mass (mg) in the in-shell test sample. The highest correlations were found between the aflatoxin concentration in ISBN samples and the aflatoxin concentration in all defective fractions (r² = 0.97). The aflatoxin mass of all defective fractions (r² = 0.90) as well as that of the rotten nut (r² = 0.88) were also strongly correlated with the aflatoxin concentration of the in-shell test samples. Process factors of 0.17,
0.16 and 0.24 were respectively calculated to estimate the aflatoxin concentration in the good kernels (edible) and good nuts by measuring the aflatoxin concentration in the in-shell test sample and in all kernels, respectively [5,6].

References
5. CONFORCAST. FINEP/MAPA, coordinator Eugenia Azevedo Vargas.

Real-world application and implementation of sampling plans for mycotoxins in pistachios

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Pistachio nuts can be infected by Aspergillus flavus or A. parasiticus, and consequently are subject to aflatoxin contamination. In California, infection most commonly occurs in nuts damaged by the navel orangeworm (NOW). Pistachio growers limit aflatoxin contamination through comprehensive NOW control programs that include orchard sanitation to reduce overwintering populations, pesticide applications timed to NOW population development, mating disruption through pheromone confusion, and early harvest to limit NOW exposure. Growers also apply non-toxigenic A. flavus to displace the toxigenic fungal strains and reduce the risk of infection by toxin-producing fungi. Despite these efforts, aflatoxin contamination still occurs. And, because it occurs within a biological system, contamination is inherently variable. Many contaminated nuts have characteristic defects and processors remove the nuts at high contamination risk through sorting processes that include airlegs, sizing, color sorters and hand pick outs. Unfortunately no sorting processes is 100% successful and a variable percentage of nuts have no easily sorted characteristic.

Pistachio processors test for aflatoxin contamination prior to shipping for most destinations including all domestic shipments and export shipments to the EU, Canada, Japan, Australia, and New Zealand. The EU/Codex sampling plan that generates 2x10 kg samples is used and generally HPLC is used for laboratory analysis, especially for exports. Tolerances or maximum residue limits (MRLs) vary with the shipment destination but pistachio processors almost always use far lower MRLs due to the variability associated with sampling for aflatoxin. The pistachio industry has worked with Drs. Tom Whitaker and Andy Slate to develop testing parameters for a variety of initial aflatoxin contamination levels that include different sample numbers and different MRLs to predict the risk of rejection at the export destination. The industry aims for a 5% or less rejection rate at the export destination. The success of pre-shipment testing is predicated on knowing the initial contamination level in finished lots. This requires thousands of tests and consequently, it is not possible to accurately estimate the initial level until about the end of January. Thus, a default testing protocol of 2x10 kg and an MRL of 3-5 μg/kg may not be appropriate for a particular crop year. Inappropriate stringency increases the cost to consumers through erroneous rejections at both ends of the shipment. The pistachio industry publishes a monthly inventory shipment report. For exports to the EU, it takes about 2 months for shipments declared in one month to arrive in the EU. Consequently, the effects of changes in testing instituted in February are not seen in the EU until April.

The pistachio industry envisions a testing program that is flexible so it can be adapted to yearly differences in aflatoxin contamination. For example, using 2x10 kg samples with an MRL of 1 μg/kg for preshipment testing for the EU was not stringent enough for the 2014 crop and testing was changed to 3x10 kg. This was done voluntarily by the handlers regulated under the federal marketing order. This change created some problems because it no longer matched the sampling protocol used in the mandatory domestic program. The inflexibility of mandatory regulations thus creates an impediment to effective and efficient testing and is a significant reason to the industry’s reluctance to create a
mandatory export program. Implementation of effective sampling plans would be facilitated by greater transparency and data sharing by both industry and the regulatory authorities.

Challenges presented by sampling and low level detection of storage molds in bulk grain

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Monitoring of chemical contaminants such as mycotoxins in bulk grain is an important measure to control the quality and safety of domestic and export shipments. Toxigenic fungi are important causal agents of mycotoxin contamination in grain and other agricultural commodities. The life cycle of fungal pathogens and storage molds is often limited to certain stages during or after plant growth, but many of their toxic metabolites are stable compounds that can still be detected long after the causal agents have retreated. In a similar manner, fragments of DNA molecules are preserved and can be used to identify and quantify microorganisms that were once actively growing on the grain. Quantitative Polymerase chain reactions (PCR) are commonly used for the detection and quantification of microbiological contaminants. In temperate climates, *Penicillium verrucosum* is causing mycotoxin contaminations in stored cereal grain.

In silos and bins, the growth of *P. verrucosum* often occurs in so called ‘hot’ spots, small pockets providing conducive conditions for the propagation of mold. The heterogeneous distribution of *P. verrucosum* and associated mycotoxins, particularly ochratoxin A (OTA), present a challenge for testing large bulk loads of grain. In addition to representative and increment sampling as well as homogenization, digital PCR (dPCR) in conjunction with TaqMan assays was used to detect single copies of the genetic markers encoding a key enzyme in the OTA biosynthesis pathway. This new technology has an advantage over other quantitative PCR methods in terms of sensitivity and precision. In this study, a droplet digital (dd) PCR system was assessed for absolute quantification of wheat samples containing traces of *P. verrucosum*. In naturally contaminated samples, successful detection and quantification of *P. verrucosum* was achieved using up to 5,000 ng template DNA per ddPCR reaction. However, sheared DNA (size 3-5 kb) for wheat samples had to be used to allow for such high concentrations of the large template DNA, which also resulted in better separation of fungal and plant clusters. Overall, the positive droplet counts of *P. verrucosum* relative to reference counts of plant DNA often correlated with levels of OTA contamination in representative and increment samples. In conclusion, the detection and low-level quantification of toxigenic fungi by droplet digital PCR can serve as a cost-effective proxy for the risk management of mycotoxin contaminations in shipments of bulk wheat.

Which sampling protocol is most cost-effective?

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Monitoring mycotoxin contamination of feed and food materials comprises collection of samples, sample preparation and chemical analyses. Mycotoxin contamination of batches tends to be concentrated in certain spots, the so-called ‘hot spots’, while the remaining part of the batch contains low(er) amounts of the toxin. The non-uniform distribution of contamination within a batch represents a great challenge to determine the true contamination level. Collection of samples is the largest source of variation associated with mycotoxin monitoring, e.g. resulting in 70-80% of the error associated with aflatoxin testing in batches of corn and peanuts. Proper sampling is thus crucial for obtaining replicable results, and is related to, amongst others, sample size, number of samples, location in the chain, sampling pattern, and sampling method. In this presentation, several studies into the most proper sampling method to apply and cost-effective methods for mycotoxin sampling will be presented. Amongst others, this will include comparison of different sampling strategies, including simple random sampling, stratified random sampling, and systematic sampling, with most appropriate numbers of samples.
Pre-harvest management strategies for mycotoxins in cereals in the USA: adapting to change

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Fusarium head blight (FHB) re-emerged in the Upper Great Plains of the U.S. with devastating impact in the early 1990s. In the two decades since those initial epidemics heralded the unprecedented resurgence of this disease, FHB has become entrenched as a chronic problem impacting the yield and quality of wheat and barley crops throughout much of the United State and has contributed to a decline in acreage of both wheat and barley. Most recently FHB also appears to have established a foothold in the Pacific Northwest, threatening wheat production in a region where it had not previously been a concern. A sustained effort, largely established and coordinately by the U.S. Wheat and Barley Scab Initiative (USWBSI), has directed research in developing control measures to minimize the threat of FHB and Fusarium mycotoxins. Significant progress has been made in developing effective disease control strategies to FHB and wheat and barley producers now have a number of tools that can help mitigate the risk of FHB and mycotoxin accumulation. Host resistance has been identified and, while immunity has been elusive, cultivars with improved resistance have been developed in all grain classes. The introgression of resistance in wheat has been aided by efficient phenotyping, achieved through the establishment of inoculated and mist-irrigated screening nurseries. Most breeding programs in the U.S. routinely use marker assisted selection, facilitated through regional genotyping laboratories, to characterize parents and/or progeny for multiple genetic markers associated with FHB resistance. The management of FHB using chemicals has also been improved through the identification of fungicides with better efficacy, along with the development of technologies facilitating the effective application of fungicides to the heads of cereal plants and by the development and deployment of a national FHB forecasting system, facilitating the use of recommended fungicides. Cooperative fungicide testing also helped to determine that the application of strobilurin fungicides may increase the risk of Fusarium mycotoxins accumulating in grain, even when applied well before flowering. Our understanding of the influence of cultural practices on the risk of FHB has also been improved. Producers are however still faced with the challenge of trying to reduce their risk from FHB by integrating numerous control strategies, as no individual measure is sufficiently effective to provide economic control. The adoption of multiple disease management practices by producers can be challenging given the various competing priorities in a wheat production system.

Cropping factors: the key for sustainable mycotoxin management in cereals – a European perspective

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To develop recommendations to avoid Fusarium head blight (FHB) and mycotoxin contamination, extensive wheat, barley and oat surveys were conducted. Furthermore, control strategies tackling crop residues as the main inoculum source are currently evaluated, including fungal antagonists and selected intercrops. For the wheat survey, harvest samples (n=686) and information on cropping measures were collected from Swiss growers (2007-2014). Grains were examined for incidence of FHB causing species, fungal DNA (qPCR), the genetic chemotype and mycotoxin content. Fusarium graminearum (FG; 65% of all isolated Fusarium species) and F. poae (FP; 21%) were dominant. The mean deoxynivalenol (DON) content was 501 μg/kg and 9% of all samples exceeded the European limit for unprocessed cereals (1,250 μg/kg). The combination of pre-crop corn and conservation tillage or ploughing resulted in an average DON content of 1,670 or 280 μg/kg, respectively, whereas the use of other pre-crops led to average DON contents of 340 and 200 μg/kg, respectively. Samples from organic farms had considerably lower FG incidence, an effect strongly linked to the high ratio of ploughed fields. The wheat variety also affected mycotoxin contamination but with different results for FG and FP. The analyses of incidence, qPCR and chemotype data revealed that nivalenol was produced by either FG, FP or F. crookwellense. The barley survey (2013-2014) showed similar patterns as those in wheat. In oats (2013-
2015), however, FP was the most dominant species and T-2/HT-2 was detected in 91% of all samples. Analyses about key cropping factors are in progress.

For growers that depend on a corn-wheat rotation, supplementary strategies are needed. Apart from fine mulching of residues to accelerate decomposition, fungal antagonists applied during corn harvest could be promising. Under controlled environment conditions, *Clonostachys rosea* performed best, completely suppressing the sexual reproduction on wheat haulms and corn stalk pieces. Currently, different formulations are examined to ensure competitiveness under field conditions. Intercrops between corn and wheat could also reduce FG inoculum, either through physical barriers or antifungal properties. Preliminary results from field experiments with three different intercrops showed that *Avena strigosa* significantly reduced ascospore release during wheat anthesis. The effect of cropping factors was quantitatively assessed and utilized to develop the DON-forecasting system FusaProg. This internet-based system employs plot-specific cropping, growth stage and regional weather data. FusaProg supports growers to manage FHB and to avoid exceeding the DON limit. It was successfully validated with more than 600 Swiss and German wheat samples.

The recently started European project MycoKey (Horizon 2020) will address mycotoxin contamination along the food and feed chain, by using a holistic approach. Integration and smart application of the above mentioned preventive and control measures could contribute to sustainable cropping systems reducing mycotoxin risks in cereals.

**Recent advances in adoption of aflatoxin biocontrol by farmers in Africa**

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A high proportion of corn and groundnuts produced and consumed in sub-Saharan Africa are contaminated with aflatoxin. Consequently, aflatoxin exposure is very high, with >95% of children below 5 years containing the toxin in their body. Aflatoxin poses a significant public health risk in many tropical developing countries and is also a barrier to the growth of domestic, regional and international markets for food and feed. Commodities containing unacceptable aflatoxin levels are either forced into low-value markets or destroyed. To minimize aflatoxin contamination in several crops, a biocontrol technology based on atoxigenic strains of *Aspergillus flavus* that do not produce aflatoxin is used widely in the U.S. Similar technology, with the generic name Aflasafe, has been adapted for use in Africa by IITA, USDA-ARS and several national partners. Aflasafe is an environment friendly granular formulation of native atoxigenic strains of *A. flavus* that are coated on sterile sorghum grains. Aflasafe application prior to crop flowering in the field displaces toxin producing *Aspergillus* strains, thereby reducing aflatoxins. Farmers in Nigeria, Kenya, and Senegal for instance continue to treat tens of thousands of hectares of corn and groundnut fields annually and have consistently achieved 80 to 99% reduction in aflatoxin contamination. The product is cost-effective since Aflasafe users are able to sell their aflatoxin-reduced corn to quality conscious food and feed industries at 13-17% premium achieving 200 to 480% return on investment on Aflasafe. Although Aflasafe deployment provides a valuable tool for promoting trade, enhancing healthier farm families, increasing income and livestock productivity and profitability, its uptake and scaling up in various African countries is hindered by lack of manufacturing, distribution and marketing capacity. Currently, only one Aflasafe manufacturing facility (production capacity: 5 ton Aflasafe/h) exists in the whole of Africa. This makes the distribution of country-specific biocontrol products in West, East and Southern African countries costly and unsustainable. In order to encourage Aflasafe use and commercialization, licensing mechanisms for manufacturing/marketing/distribution needs to be in place. The Bill & Melinda Gates Foundation and USAID have recently funded a technology transfer and commercialization initiative to scale-up use of Aflasafe in 500,000 ha in 11 African nations through private, public, or public-private partnerships.
The role of endophytes in biological control of toxigenic fungi: new hope for success?

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Endophytes are microbes that inhabit internal plant tissues without causing disease. Some endophytes can protect their hosts against pathogens. We hypothesized that plants which grow without synthetic pesticides, including the wild and ancient relatives of modern crops, and the marginalized crops grown by subsistence farmers, host endophytes that have co-evolved to combat host-specific pathogens. To test this hypothesis, we explored endophytes within the ancient Afro-Indian crop finger millet, and diverse corn/teosinte genotypes from the Americas, for anti-fungal activity against Fusarium graminearum. F. graminearum leads to Fusarium head blight (FHB) in wheat and Gibberella ear rot (GER) in modern corn and is associated with accumulation of mycotoxins including deoxynivalenol (DON). Here we report that bacterial endophytes from both wild/ancient corn and finger millet can efficiently suppress F. graminearum in modern corn and wheat and dramatically reduce DON during seed storage. The most exciting discovery is that an endophytic bacterium (strain M6, Enterobacter sp.) isolated from the roots of finger millet builds a remarkable physical barrier consisting of bacterial microcolonies that protect the host against pathogen invasion. M6 creates an unusual root hair-endophyte stacking (RHESst) formation that prevents entry and/or traps the pathogen which is then killed. Tn5 mutant analysis demonstrates that the endophyte kills the fungal pathogen by diverse natural fungicides, including phenazine. The end-result of this remarkable physico-chemical barrier is a reduction in levels of DON mycotoxin, thus potentially protecting millions of subsistence farmers and their livestock. Our research suggests the value of exploring the microbiomes of the world's wild plants and orphan crops as sources of endophytes with antimicrobial activity.

The effect of corn plant density and water availability on fumonisin producing Fusarium spp. infection and fumonisin synthesis

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Corn (Zea mays L.) is one of the most important crops worldwide, and is a host to many plant pathogens, including Fusarium verticillioides. This fungus produces fumonisins, which cause mycotoxicoses in animals and humans when ingested. The aim of this study was to investigate the effect of plant density and water availability on F. verticillioides ear rot infection and fumonisin contamination under field and glasshouse conditions. Field plant density trials were planted at the ARC-GCI from 2011-2014 and comprised of 10,000, 20,000, 30,000, 40,000 and 50,000 plants per ha using cultivars CRN3505 and PAN6P-110 in a completely randomized block design. Glasshouse watering regime trials (2012-2015) comprised of 15-, 20-, 25-, 30- and 35 l water per week. Cultivars CRN3505 and PAN6P-110 were planted in 80 l plastic bags and watered with buckets. Ears were inoculated at silk stage with high fumonisin producing isolate MRC826. Grain from individual field and glasshouse trials were milled and subjected to qPCR (target DNA quantification) and HPLC (fumonisin quantification). Analysis of variance indicated a highly significant interaction (treatment x season x cultivar) regarding fungal infection (p<.001) with the plant density field trials. Target DNA decreased as plant densities increased. Mean target DNA increased from 2011-2014 with CRN3505 having a lower mean target DNA compared to PAN6P-110. A weak treatment x season interaction was observed for fumonisin synthesis (p=0.06). Similar to fungal biomass, fumonisins decreased as plant densities increased although target DNA increased over seasons and fumonisins decreased. In the watering regime glasshouse trials, analysis of variance indicated a significant treatment and cultivar effect with respect to F. verticillioides infection (p=0.01). Fungal infection decreased as watering regime increased. CRN3505 had significantly higher mean fungal infection and fumonisin synthesis compared to PAN6P-110, possibly due to the glasshouse trials being inoculated as opposed to natural infection in field trials. This research could enable corn producers to obtain desired yields with reduced fungal infection and fumonisin contamination in grain.
Ergot management solutions: an integrated approach in Western Canada

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Ergot, caused by the ascomycete fungus Claviceps purpurea (Fr.) Tul., has increased as a problem in wheat grown on the Canadian prairies since 1999. Levels of ergot infestation of common wheat delivered to elevators rose from less than 1% of all deliveries in 2002 to infestation levels of 50%, 25% and 30% of all deliveries in Alberta, Saskatchewan and Manitoba respectively in 2013. Losses to producers resulted when their wheat was either downgraded or refused delivery at the elevator, or through costs accrued during attempts to clean the grain, as well as raised concerns of ergot alkaloid contamination of human food or animal feed. Management of ergot infection of wheat crops in Western Canada is not based on a sole control practice, but requires an integrated approach. Management practices that can influence ergot severity and infestation of grain include crop rotation, management and nutrition, pesticide applications, and harvest or post-harvest techniques for reducing ergot in the grain. Although small differences exist among wheat varieties and classes, there is little genetic resistance among current wheat varieties to this pathogen.

Cisgenic wheat, through genome editing, to reduce Fusarium mycotoxins in grains

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Crop plants suffer from several devastating plant diseases, causing not only severe yield losses but also produce mycotoxins in the consumed products. Several postharvest technologies can reduce toxins but these are often difficult or not enough. The pathogen Fusarium graminearum infects wheat and barley spikelets and accumulate trichothecene mycotoxins in grains. The regulatory authorities limit the toxin content to be 1-5 mg/kg depending on human or animal use. Genetic resistance in plants is considered to be the best way to manage this disease and the mycotoxins in grains. The resistance is due to hierarchies of R genes that eventually produce resistance related (RR) metabolites and proteins [1]. Candidate R genes can be identified in world germplasm collections or in resistant cultivars. These candidate R genes can be sequenced in commercial cultivars, and if nonfunctional, they can be replaced with functional genes, from a resistance source genotype from a sexually compatible species; the resulting plant is generally designated as cisgenic cultivar. Several genome editing technologies are available for gene replacement but not all are considered safe by the regulatory authorities. Replacement of candidate R genes/segments using ribonucleoprotein in a CRISPR system, by directly introducing it to protoplast, is considered safe as it replaces the gene in exactly the same genomic location and also it does not leave any foreign DNA in the cisgenic cultivar. However, the public generally do not distinguish genetically engineered from genetically modified with foreign gene, crops. They have to be educated by researchers, industries and the government as well.

References

Support from modeling in climate change scenarios

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There are several certainties on climate change scenarios, like the increase in air temperature and CO2, different rain distribution along the year, with increases in rainy days and rain intensity during winter and decreases in summer time. Besides, more extreme events are expected, like very high temperature or exceptional amount of rain fallen in a short time. Accordingly, different biogeography of plants and fungi is expected, with some confirmations published. On the other side, there are several unknowns, like annual weather conditions or extremes in sub-periods during each crop season. Overall, climate change
is considered to increase health risks and mycotoxins play a pivotal role in this context. Fungi (and related toxins) of main concern are expected to change between years and during each year, with the interaction between toxigenic fungi acquiring increasing relevance. Corn in central-southern Europe is an interesting example. Corn is commonly contaminated by fumonisins, frequently above the legal limit for products destined to human consumption. The first outbreak of severe aflatoxin contamination in southern Europe happened in 2003, followed by points of high contamination in the following years and 2 further serious events in 2012 and 2015. During the last 12-year period, 2014 showed high deoxynivalenol contamination and 2011 was a safe year, with very low detection of all mycotoxins. In this context of uncertainty, predictions become essential in preventing mycotoxin to enter the food and feed chains; crop phenology, weather and mycotoxins all need support from predictive modeling. Predictive models allow to generate, in the next or far future, weather data, to predict crop geographic distribution and phenology and to draw mycotoxin risk scenarios. Mycotoxin prevention is based on cropping tactic choices, most of them placed early in the season, before crop became susceptible to toxigenic fungi. Nevertheless, predictive models, with actual/historical meteorological data as input, are considered useful tools. They can support farmers, extension services and stakeholders to rationalize pre- & post-harvest crops and products management, highlight risk areas and, therefore, help in sampling and analysis optimization, making mycotoxin management more sustainable and efficient. Risk managers, policy makers, Institutions and researchers can receive support from models fed with climate change data to define emerging risks and try to answer to the open question ‘will mycotoxin contamination be more severe in climate change scenarios?’. Experiences are still limited, but really interesting and useful for developing strategies to better face future risk scenarios.

Is organic farming a suitable approach to reduce mycotoxins in agricultural crops?

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This paper presents peer-reviewed studies comparing the content of deoxynivalenol (DON), HT-2+T-2 toxins, zearalenone (ZEN), nivalenol (NIV), ochratoxin A (OTA) and fumonisins in cereal grains, and patulin (PAT) in apple and apple-based products from organic and conventional production in temperate regions. Some of the studies are based on data from controlled field trials, however, most are farm surveys and some are food basket surveys. Comparison of the results between different studies are complicated due to differences in sampling strategy, different reporting of the results, different use of statistics, different limits of detection and quantifications etc. Almost half of the studies focused on DON in cereals. The majority of these studies found no significant difference in DON content in grain from the two farming systems, but some studies reported lower DON content in organically than in conventionally produced cereals. Many authors suggested that weather conditions, years, locations, tillage practice and crop rotation are more important for the development of DON than the type of farming. Organically produced oats contained mainly lower levels of the sum of HT-2 and T-2 toxins than conventionally produced oats. Most studies on ZEN reported no differences between farming systems, or lower concentrations in organically produced grain. For the other mycotoxins in cereals, mainly low levels and no differences between the two farming systems were reported. Some studies showed higher PAT contamination in organically than in conventionally produced apple and apple products. The difference may be due to more efficient disease control in conventional orchards. Based on the available information, it cannot be concluded that any of the two farming systems increases the risk of mycotoxin contamination. Despite no use of fungicides, an organic system appears generally able to maintain mycotoxin contamination at low levels. More systematic comparisons from scientifically controlled field trials and surveys are needed to clarify if there are differences in the risk of mycotoxin contamination between organically and conventionally produced crops.
Validation of the planar waveguide biosensor for multi-mycotoxin analysis of wheat

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The Bayer Quality Analyzer is a commercially available system for screening deoxynivalenol, ochratoxin A, zearalenone, and T-2 toxin in wheat. Analysis is based on a competitive immunoassay, and uses planar waveguide technology for the detection of mycotoxin/fluorescently-labeled antibody complexes. A two stage evaluation of the biosensor method was performed. A multi-day evaluation of accuracy and precision using incurred samples and certified reference materials was completed followed by the analysis of samples previously analyzed by instrumental methods. The samples analyzed represented a variety of Canadian wheat classes. Method performance was good around concentrations of interest for compliance testing. Around existing regulatory limits for deoxynivalenol (1,250-2,000 ng/g) and ochratoxin A (5 ng/g), the recoveries and repeatability met performance criteria outlined in European Commission Regulation No. 401/2006. Unfortunately, there were not enough appropriate samples to confidently evaluate the performance of the method for the analysis of zearalenone and T-2. In general, the performance of the biosensor method during the analysis of wheat met the EU criteria for deoxynivalenol and ochratoxin A analysis. The main advantage of the biosensor method is the multi-mycotoxin capability as the most relevant mycotoxin analytes for Canadian wheat – deoxynivalenol and ochratoxin A – are covered in one run. This saves personnel time.

Dried blood spots: a powerful tool to analyze mycotoxins in blood samples

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The risk assessment of mycotoxins in general is mainly based on toxicity data from animal experiments in combination with reliable data of mycotoxin occurrence and food consumption data. Based on this approach limits and regulations for mycotoxins in food samples are established in many countries. One major drawback of such risk assessment is a potential underrepresentation of subgroups with a regional, cultural or medically-determined deviation in their nutritional habits, hence resulting in an altered exposition than estimated based on global consumption databases. To overcome this problem, the individual exposure assessment based on the analysis of physiological samples would be the method of choice and could significantly contribute to an improved risk assessment. Limitations in the analysis of physiological samples, especially blood samples, are the laborious and time consuming sample collection and cleanup.

Within the last years the use of dried blood spots (DBS) gained more and more attention due to the development of new applications, besides the well-known use in clinical laboratories. In this paper, we present for the first time the use of dried blood spots in combination with highly sophisticated HPLC-MS/MS instrumentation of the newest generation for the analysis of mycotoxins as well as their metabolites in blood samples. In order to evaluate the possibilities and limitations in mycotoxin analysis ochratoxin A (OTA) was used as model compound. The DBS technique was advanced for the analysis of OTA in DBS with unknown amounts of blood as well as varying hematocrit values. Furthermore, the comparability of venous vs. capillary blood was investigated [1,2]. The results show that 20 µl of human blood taken from finger-pricks are sufficient to quantify OTA at levels as low as 0.026 ng/ml (LOQ) with just one extraction step for cleanup using a mixture of acetone, acetonitrile and water. OTA concentrations of the blood samples from fifty volunteers from Germany ranged between 0.07 ng/ml to 0.38 ng/mL with a mean value of 0.21 ng/ml. In the case of coffee consumption the isomer 2’R-ochratoxin A, which is formed during coffee roasting, was also detectable with a mean value of 0.11 ng/ml [1]. The complete validation of a multi-mycotoxin method for the analysis of dried blood spots is currently under study and will be presented as well.

References
Progress and challenges in aptamer-based mycotoxin sensing

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Aptamers are single-stranded oligonucleotides with the ability to bind tightly and selectively to a target analyte. High affinity and specific aptamers for a variety of mycotoxins have been reported over the past decade. Increasingly, these molecular recognition elements are finding applications in biosensors and assays for the detection of mycotoxins in a variety of complex matrices. This presentation will highlight the mycotoxin aptamers that are available for mycotoxin detection and select assays and biosensing platforms into which they have been incorporated. In particular, aptamer systems for ochratoxin A and fumonisin B1 will be examined in detail [1,2]. Key advantages that aptamers have over analogous technology, and areas where these advantages may be applied for the benefit of practical mycotoxin detection, will be discussed. Bottlenecks in the application of aptamers, including technical challenges in the selection and characterization of mycotoxin aptamers, will be examined [3]. Given the robust nature of aptamers and the low cost of their synthesis, they are uniquely suited as components for the development of robust, inexpensive, portable mycotoxin assays or detection technology. Progress on several aptamer-based platforms will be described ranging from paper-based assays [4] to a lab-on-a-chip chemiluminescent aptasensor system [5].

References

Optimization and validation of LC-MS/MS based methods for the simultaneous determination of hundreds of fungal metabolites

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In the recent years, LC-MS/MS based multi-mycotoxin methods have become more and more popular as they are able to simultaneously cover all regulated toxins. As the application of sophisticated methods for sample cleanup is ruled out by the chemical diversity of this set of analytes, most of the related methods rely on some sort of unspecific cleanup (e.g., modified QuEChERS) or on isotopically labelled internal standards. However, none of these approaches is fully applicable, if the range of analytes is further extended. Therefore, our method targeting several hundreds of fungal metabolites and a few plant toxins is based on the direct injection of diluted crude extracts. Many authors have expressed their concern about the limited accuracy of this approach, as matrix effects might not be effectively under control. Indeed, the decrease (or more rarely the increase) of the analytical signal due to co-eluting matrix constituents is the most critical issue that needs to be minimized (by using a low injection volume in connection with a large flow rate) or thoroughly investigated during method validation. However, there is still a lack of related guidelines as matrix effects are sometimes not specifically mentioned in official documents (e.g., Commission Decision 2002/657/EC) or are addressed by guidance documents set for other types of contaminants (such as SANCO/12571/2013 for pesticide residues). In our view, the aspect that needs particular attention is the number of different individual samples per matrix that should be investigated to determine the variation of matrix effects within a given matrix. This is very often neglected by methods described in the literature that use matrix calibration matched to a single or a pooled blank sample. In relation to this, it is still to be discussed whether apparent recoveries that are low but consistent (i.e., demonstrating good precision) may be acceptable in multi-analyte analysis.
Safe foods from farm to table: how IR laser technology and chemometrics spot trace level mycotoxins

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Infrared spectroscopy is now a well-established method for the determination of major components in food and feed, e.g., for the measurement of protein and moisture content. Data analysis is usually carried out employing a spectral window for multivariate statistical analysis of data (i.e., chemometrics). The objective of the EU-funded FP7 MYCOSPEC project was to extend these capabilities to minor and trace components such as mycotoxin contamination in corn and peanuts. Small spectral changes caused by changes in protein and carbohydrate content due to fungal contamination, were employed to classify contaminated and clean samples at EU legal limits; i.e., 500 µg/kg for deoxynivalenol in corn and 8 µg/kg for aflatoxin B1 in peanuts. A large number of mid-IR spectra of corn (n=184; concentrations <LOD to 50,160 µg/kg), and peanuts (n=92; <LOD to 10,624 µg/kg), naturally and artificially infected with fungal species were generated. Deoxynivalenol and aflatoxin B1 reference contaminations were assessed using a validated liquid chromatography-mass spectrometry method. Bagged decision tree classification and principal component analysis models were built and cross-validated with classification accuracy reaching 86% (corn) and 77% (peanuts), resulting in successful classification in the low µg/kg range for commercial peanut samples at legally relevant limits. Results, including investigated spectral windows served as a basis for quantum cascade laser based measurements and modeling of laser spectral data aimed at increasing sensitivity even further.

Entering the ppq level in mycotoxin biomarker analysis

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There is increasing awareness of the need to understand the patterns and levels of mixtures of dietary mycotoxins. To achieve this goal, mycotoxins and their metabolites are being measured in urine. We previously published a multi-mycotoxin LC-MS/MS survey of 120 Nigerian urine samples, however we were aware that in measuring the mixture a compromise on assay sensitivity was incurred compared to some established single-analyte methods. In order to allow more accurate and comparative dietary mycotoxin exposure determination, a highly sensitive urinary assay, including 13C-labeled or deuterated internal standard, was developed and validated for seven important mycotoxins (and several metabolites) including aflatoxin M1 (AFM1), fumonisin B1 (FB1), ochratoxin A (OTA), deoxynivalenol, deoxy-deoxynivalenol, nivalenol, zearalenone (ZEN), citrinin, dihydro-citrinin, α- and β-zearalenol, and alternariol. The assay includes β-glucuronidase pre-treatment, SPE enrichment, UHPLC separation and high precision triple quadrupole MS/MS detection. Analytical sensitivity was significantly improved, providing parity or better compared to existing single analyte methods, but in a single more robust multi-mycotoxin assay. On re-analysis of the Nigerian urine samples there was an increased number of positive samples for all tested mycotoxins: from 50.8% to 100%, with highest occurrence of ZEN (82%), OTA (76%), AFM1 (73%), and FB1 (71%). Overall, a significant share of samples (68 out of 120; 57%) were contaminated with both AFM1 and FB1.
Electrochemical immunochemical techniques for rapid screening of mycotoxins in different matrices

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Food-related risks can spread easy as a result of the globalized ingredients transport. Therefore, it is of high importance to control microbiological and chemical hazards in order to guarantee food safety. About 25% of all crops worldwide are affected by mycotoxins. This has a great impact on food and feed production and livestock farming, holding an increased security risk for human health. Mycotoxins, secondary metabolites produced by various fungal species, can be present in feed products and food at very low concentrations (ppb-ppt range). However, depending on the type of mycotoxins, they can cause serious toxic effects in humans and animals. Additionally, mycotoxin concentrations increase during storage and transport. Therefore, mycotoxin investigating is of high importance. Moreover, reducing the mycotoxin contamination risk by controlling through rapid, sensitive and accurate analysis is highly needed.

The assessment of mycotoxins exposure is often based on the combination of chemical analysis of foodstuff and correlation of the obtained results with population data on food consumption. Risk assessment of mycotoxins can be also evaluated by the determination of biomarkers. Mycotoxins’ urinary biomarkers have chromatographic origins and, therefore despite their undoubted high specificity and sensitivity, quite expensive, time-consuming and ecologically unfriendly. Nowadays the amount of publications covering easy-to-operate immunochemical tests for simultaneous determination of several analytes is constantly rising. Different labels have been developed for application in immunochemical assays in order to reach the highest possible sensitivity. Quantum dots (QDs) have been widely used in diagnostics, drug delivery research, biolabeling and chemical analysis as promising fluorescent labels for imaging and quantitative detection. Application of QDs enables the simultaneous detection of multiple analytes on one single spot provided their conjugates are labeled with QDs which are fluorescent in different parts of the spectrum. In this work, a number of screening approaches (fluorescent immunosorbent assay, lateral-flow test, membrane-based...
test) for single and multiple detection of mycotoxins in various matrices from cereals to urine were developed for holistic estimation of mycotoxins exposure with followed confirmation by LC-MS/MS.

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Real time monitoring of stored commodities for better post-harvest mycotoxin management

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Effective harvesting and storage of food/feed commodities is critical to avoid mold spoilage and mycotoxin contamination, especially in a global market where they are transported across climatic zones over long distances. Losses post-harvest can be significant (5-25%) impacting both economically on value of the commodity and on the nutritional value. These losses may be further exacerbated by climate change. Many stored commodities are alive and thus respire during storage. While temperature and relative humidity are often monitored periodically or on line in silos for stored commodities, carbon dioxide (CO₂) production is a more sensitive and early indicator of the activity of both molds and pests. Thus it is possible to integrate physical data in real time with biological data and models on the boundary limits of moisture content and temperatures for growth and mycotoxin production by key mycotoxigenic fungi. This could be used for the development of an effective post-harvest decision support system (DSS) for better management of stored commodities, especially cereals, tree nuts and other foodstuffs.

By integrating data on wheat contaminated with *Penicillium verrucosum* and ochratoxin A and corn with *Fusarium verticillioides* and fumonisin B1 for CO₂ production and dry matter losses under different temperature x moisture content conditions and the biological boundary conditions for growth and mycotoxin production, potential for the development of a DSS system exists. This could have significant benefits for post-harvest management of such commodities and minimize both mycotoxin contamination and quality/nutritional losses.

Sorting of mycotoxin-contaminated products with hyperspectral imaging

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Imaging is the process of digitizing the light reflected from objects. The compounds in objects have different absorption and reflection properties, and they reflect/transmit light at different wavelengths. Hyperspectral imaging (HSI) is the process of imaging the objects at different spectral bands. By analyzing the spectral behaviors of the acquired image, we can get an idea about the reflecting structure of the object. HSI is effectively used in agriculture in the fields (to monitor the crops, different vegetation, etc.) and in mining and geology (to observe various minerals). HSI is also used in the food industry to detect contaminated food items non-invasively. Agricultural products (figs, nuts, corn, wheat, etc.) are prone to mycotoxin producing molds; mycotoxin-contaminated food should be eliminated before marketing. Mycotoxin producing molds affect the structure of agricultural products changing the reflected/transmitted light. If the mold diffuses into the food, the spectral signature indicating the presence of mycotoxin can be obtained from transmitted light whereas, if the mold causes structural effects at the outer shell, the spectral signature is observed from reflected light. Using HIS, aflatoxin-contaminated hazelnuts and dried figs are detected by reflectance images with high classification accuracy. Ochratoxin can also occur in dried figs; molds producing ochratoxin are usually present inside the figs. The figs with ochratoxin inside may not be imaged by reflectance. They are imaged non-invasively using transmittance images at different wavelengths and automatically checked considering the spectral signatures.

Decontamination and detoxification of mycotoxins in grain to improve feed quality and animal health

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It has been estimated that two million metric tons of wheat and durum grown in Western Canada in 2014 were graded as salvage (having no value) due to high levels of *Fusarium*-damaged kernels (FDK) and potential for *Fusarium*-related mycotoxins, such as deoxynivalenol (DON). Only very small amounts of DON (<1 mg/kg) are tolerated in grain destined for human consumption. In Canada, the maximum...
tolerated level for DON in animal feed is between 1-5 mg/kg depending on the species while higher levels are associated with negative performance and health impacts in animals. Therefore, in years when there is limited high quality grain, a greater proportion of downgraded grain will be diverted to animal feed. Besides the obvious risk to animal health, this situation also means a significant economic loss to producers. In order to recover lost value of downgraded and salvage grain, we need to develop methods to effectively reduce FDK and associated mycotoxins as well as find a way to detoxify and utilize salvage grain. Large-scale individual kernel sorting using near infrared transmission (NIT) technology has demonstrated significant capacity to sort downgraded FDK wheat sources into higher quality fractions. We are using this technology to produce naturally contaminated diets from a single grain source for studies on mycotoxicosis in animals. For example, we have demonstrated the efficiency of NIT in sorting three different class of Canadian wheat containing FDK and then used these sorted fractions to formulate four levels of mycotoxin-contaminated diets to assess how highly contaminated grain fractions obtained by sorting can be used in dietary exposures with broilers chickens. In another study we are using NIT to produce varying levels of *Fusarium*-contaminated grain (including >10% FDK=salvage) to determine if insects are able to utilize FDK salvage wheat as a food source without accumulating the mycotoxins. If we can successfully convert highly contaminated material into insect larvae with high protein and fat, we could set up a market for salvage wheat and in the long term, provide the feed industry with a more sustainable and cheaper source of crude protein for animal feed.

**Corn grain ensiling is a process conducive to fumonisin B1 degradation: which microbial consortia are responsible?**

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Use of high moisture corn grain silages in diets of ruminants and monogastric animals has significantly increased in Europe during the past decade. What happens to field mycotoxins accumulated in grains during ensiling has however never been documented. In this study, an optimized procedure that allows assessing both free and matrix-associated fumonisin B1 (FB1) forms was defined and used to investigate the fate of FB1 during the fermentation of moist corn grain silages in small scale silos. Our results showed that, whatever the initial amount of FB1 in corn grains, a significant decrease in FB1 content occurred. It reached as much as 75% after 4 months of fermentation in certain silages. In addition, our data demonstrated that the decrease in toxin content was mainly due to degradation processes, which was also supported by the detection of partially hydrolyzed FB1 in some fermented samples. A comparative genomic strategy was further undertaken with the aim of identifying the fermentative microbial consortia that could be associated with FB1 degradation. A selective microbial DNA extraction procedure coupled with high-throughput shotgun sequencing was optimized. Preliminary results showed specificities of consortia in FB1-degrading vs. FB1-non degrading silages.

**Distribution of deoxynivalenol in wheat during milling**

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Deoxynivalenol (DON or vomitoxin), a trichothecence mycotoxin, is mainly produced by *Fusarium graminearum* and its related fungi. As a plant pathogen, *F. graminearum* can infect growing crops in the field to cause *Fusarium* head blight (FHB) particularly in wheat and barley which leads to DON contamination. In addition to a significant economic impact, DON contamination is a serious concern in human and animal health due to its adverse effects including diarrhea, vomiting, gastro-intestinal inflammation, and immunomodulation. DON present in a kernel is mainly distributed toward the outer layer including bran. Therefore, physical treatment, such as milling, can only redistribute the toxin into different fractions with varying concentrations rather than removing it from the product. Nonetheless, DON levels in certain milled fractions such as flour can be reduced substantially by removing bran and germ from the infected kernels. Sorting and mechanical cleaning prior to milling may reduce DON concentration significantly as shriveled or heavily infected kernels with high concentration of DON can be rejected from the product stream.
Impact of processing techniques on mycotoxins occurrence in food

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Spoilage and toxin formation can occur in the field, during storage of agricultural commodities and the processing of foodstuffs. A variety of fungal species mostly from the genera Aspergillus, Penicillium, Fusarium, Alternaria or Claviceps can produce mycotoxins. Most important in terms of toxicity and occurrence are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and other trichothecenes, zearalenone, patulin and ergot alkaloids. Good agricultural practices, plant diseases management and adequate storage conditions reduce mycotoxin levels in the food chain, yet do not eliminate mycotoxins completely. In 2014, the International Life Sciences Institute Europe (ILSI Europe) Process-related Compounds and Natural Toxins Task Force started a project dedicated to understand the possibility in mitigating mycotoxins, correspondently improving the safety of the food commodities. The main task defined was to critically review the state of the art about mycotoxin reduction by food processing and this activity can be now considered almost completed.

In fact, in order to prevent losses of otherwise suitable feed materials and foodstuffs due to mycotoxins, decontamination processes have been developed and applied over the years. Food processing can impact mycotoxins in raw material in different ways: (1) physical elimination (e.g., cleaning, sieving); (2) chemical transformation or interaction with food matrix which can result in metabolites of either lower or higher toxicity than the parent compound; (3) release from masked or entrapped forms which may increase bioavailability; (4) enzymatic detoxification; and (5) adsorption to bacterial cell walls which may be reversed during further processing or digestion. The objective of this presentation is therefore to give an overview about the aspects indicated above and the correspondent work carried on by the ILSI dedicated expert group, targeting worldwide solutions scientifically available. Gaps in knowledge will be identified; the need for prioritization of further research, as well as for risk assessment procedures with regard to product safety for consumers will be discussed.

Promising detoxification strategies to mitigate mycotoxins

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Yearly surveys demonstrate that mycotoxin contamination of foods and feeds represent a worldwide public health issue. Moreover, analytical progress demonstrated that this problem could be more important than previously estimated due to the presence of masked and/or modified toxins that were not taken into account. Finally, global climatic changes also make the situation more complex, leading to the appearance of known toxins in areas that were considered as not present (i.e., aflatoxin B1 in corn in Central Europe). Despite the identification and publication of relevant good agricultural practices in official guides, the observed levels of contamination demonstrate that this approach is not sufficient to ensure food safety. In the same way, the use of pesticide to control the development of toxigenic fungi is also debatable due to the appearance of resistance in target organisms and the toxicity of these products and the strong societal will to reduce their use in Europe. Therefore, there is a stringent need to look for new strategies to limit mycotoxin contamination. Recent progress in the knowledge of fungal eco-physiology and mycotoxin metabolism allowed the development of new promising approaches to limit both human and animal exposure to these contaminants.

The first approach aims to limit mycotoxin synthesis. For that, the use of atoxigenic strains and competing organism has been extensively studied. Another promising approach is to identify natural compounds able to specifically inhibit toxin production without interfering fungal development. This approach would allow ensuring food safety without perturbation of biodiversity and limiting the risk of emergence of new hazards. Some plant extracts were demonstrated to inhibit toxin production, mostly aflatoxin B1, with limited impact on fungal development. Although their mechanisms of action were only
rarely precisely identified, it seems that they could act at transcriptional level. The identification of the cellular signals that lead to down modulation of the toxin production is now of importance to screen new natural compounds on the basis of a clear structure function relationship and to identify the key points of fungal secondary metabolism to target to block toxin production. The second important stage were mycotoxin toxicity can be mitigated is later, directly in the intestine by modifying absorption or transforming toxins into non-toxic compounds.

**Management of food industrial technologies reducing mycotoxins while keeping the quality of finished products**

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In 2014, the International Life Sciences Institute Europe (ILSI Europe) Process-related Compounds and Natural Toxins Task Force started a project dedicated to understand the possibility in mitigating mycotoxins, correspondently improving the safety of the food commodities. The main task defined was to critically review the state of the art about mycotoxin reduction by food processing. The various food processing that may have mitigating effects on mycotoxins include cleaning, washing, steeping, milling, brewing, fermentation, cooking, baking, frying, roasting, flaking, nixtamalization (alkaline cooking), and extrusion. In order to reduce the high levels of mycotoxins, addition of a number of chemicals has been studied: acids, alkaline agents, oxidizing and reducing agents which most of the time reduce the mycotoxin level but have a negative effect on the nutritional value, taste and color of the final product.

In currently applied food grade industrial processes, chemical agents are added in an adapted way to steer these processes to provide for food ingredients as corn flour for the production of masa, corn wet milling to provide for food grade starch and crude oil processing to refined vegetable oils. The objective of this presentation is to give an overview about the effect of a number of chemical agents that provide for metabolites and their fates in a number of large scale food processes. The following examples of reduction of mycotoxins will be described in this presentation: (1) nixtamalization of corn in production of masa; (2) reduction of *Fusarium* mycotoxins in corn starch obtained through corn wet milling; and (3) removal of aflatoxins in refinement of vegetable oils.
Taxonomy of fungi producing emerging mycotoxins

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DNA-based phylogenetic analysis improves taxonomic understanding of mycotoxigenic fungi by making species concepts less subjective and by clarifying evolutionary relationships. The shift from dual nomenclature, where sexual and asexual states of one species were classified in different genera, to a system where one fungus has only one name, eventually will simplify fungal taxonomy but is now causing some confusion. Controversies over circumscription of genera exist for Alternaria, Fusarium and Aspergillus, but except for the latter, would not affect the names of mycotoxigenic species.

Eurotiales. In the Aspergillus clade (~345 spp.) aflatoxins are a major concern for the A. flavus group (22 spp.), ochratoxin A (OTA) for the A. ochraceus group (27 spp.), OTA and fumonisins for the A. niger group (25 spp.), while most species pathogenic to humans and animals are in different clades. Penicillium has been divided into two genera, Penicillium (~380 spp.) and Talaromyces (~105 spp.), with most major mycotoxin and antibiotic producing species remaining in Penicillium. OTA is produced by a clade of three closely related species, i.e., P. verrucosum, P. nordicum and P. thomicola. Citrinin is produced by species of the P. citrinum group (16 of 39 spp.), but both citrinin and patulin production are broadly scattered in Penicillium, Aspergillus and related genera. ITS sequences are the primary barcodes for these genera, with precise species identification requiring a secondary barcode, i.e., calmodulin for Aspergillus, and β-tubulin for Penicillium and Talaromyces. Lists of accepted species, including reference barcodes, for all three genera are published and updated at www.aspergilluspenicillium.org.

Hypocreales. Taxonomists working with Fusarium led the application of multi-gene phylogenetics to phylogenetic species recognition, but have not produced lists of accepted species or reference barcodes. Fumonisins are produced by a few species of the F. fujikuroi complex and have so far otherwise have only been found in some strains of the F. oxysporum lineages. Trichothecene producing mycotoxin species occur in a clade including five species complexes. The F. graminearum species complex now comprises 15 phylogenetic species producing similar toxins; the main biochemical variants (3- and 15-acetyldeoxynivalenol, nivalenol, NX chemotypes) of concern in North America are all within F. graminearum in the strict sense. Several emerging mycotoxins differ in biosynthetic origin, but are produced by the same or other species of the trichothecene clade of Fusarium. ITS barcoding is seldom used in Fusarium because of a lack of resolution and the existence of paralogues, and identifications compare either TEF-1a or RPB2 sequences to data on isolate.fusariumdb.org. In comparison with most mycotoxigenic fungi, the ergot genus Claviceps, known for its potent alkaloids, has received surprisingly little modern taxonomic attention and is the topic of another presentation in this symposium.

Pleosporales. A morphological monograph of Alternaria published in 2007 included ~300 species. Multigene-phylogenies and genomic comparisons later led to the collapse of 35 entities formerly considered species, into a broad species concept for A. alternata. The genetics of this ‘species’ is complicated by the existence of non-mitochondrial plasmids involved producing host-specific toxins. To date, only one strain isolated from tomato has been shown to produce the fumonisin-like AAL toxin. Other emerging mycotoxins, such as alternariol, are produced by many strains of A. alternata. ITS barcodes are used for preliminary identifications of Alternaria isolates, with GAPDH and endoPG genes recommended as secondary barcodes. No list of accepted species or reference barcodes has been published.

Full genome sequencing is now beginning to affect the taxonomy of mycotoxigenic fungi, especially in Alternaria, Aspergillus, and Fusarium. This allows us to envisage a time within 5-10 years when simultaneous identification of both species and mycotoxigenic capacity will be possible and routine.

Non-targeted screening as a method for profiling mycotoxins

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Emerging mycotoxins of concern including conjugated mycotoxins are frequently being identified, necessitating the need to develop analytical methods in order to measure the exposure risk to
consumers. Data obtained by traditional targeted LC-MS/MS methods cannot be mined for these new compounds of concern and samples must be re-analyzed. In response, we developed a liquid chromatography-data independent acquisition (LC-DIA) method on a Q-Exactive Orbitrap mass spectrometer tailored specifically for mycotoxins analysis that combines absolute quantification of targeted fungal metabolites with non-targeted digital archiving (DA) of data on all ionisable compounds for retrospective analysis. We have applied this method to quantify known mycotoxins in Fusarium infected corn, ensiled corn and store bought foodstuffs all while storing the data in a searchable digital archive allowing for retrospective analysis of newly reported compounds. In 2015, Schmeitzl et al. [1] reported on the production of 15-acetyldeoxynivalenol-3-O-β-D-glucoside by wheat suspension cultures treated with 15-acetyldeoxynivalenol and provided a high resolution MS/MS spectrum. Using this information, we retrospectively analyzed our previously acquired data and detected this recently reported conjugated mycotoxin in corn, which was naturally infected by the 15-acetyldeoxynivalenol chemotype of F. graminearum without requiring re-analysis of the samples. To our knowledge, this was the first reported occurrence of this conjugated mycotoxin in naturally contaminated corn, demonstrating the utility of using a single screening method to quantify known mycotoxins and archive a completely non-targeted dataset for retrospective analysis.

References

Using yeasts to study mycotoxin toxicity and interactions

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Yeast species are ideal model organisms to study the toxicity, mode of actions, cellular interactions and effects of mycotoxins. As a well characterized model eukaryotic organism, yeast (Saccharomyces cerevisiae) has many advantages over more complex multicellular models. Yeast has a sequenced genome with Gene deletion collections and tagged protein expression collections. They have been adapted for two-hybrid interactome analysis, while gene replacement methods abound. These extensive genomic and proteomic resources permit rapid development of real-time assays to study the effects and consequences of toxins both singly, and in combinations as well as fungal filtrates or secretomes. We have conducted numerous studies to measure the effects of single amino acid substitutions in a protein thought to be the target of mycotoxins (ribosomal protein L3). We will highlight results from studies using (1) novel antibodies directed against mycotoxins, (2) the yeast knock-out collection to search for novel targets of mycotoxins, and (3) yeast with modified transporter capabilities to identify and alter cellular exporters of mycotoxins.

Discovery of novel Fusarium secondary metabolites

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The genome of Fusarium graminearum contains a significant number of both clustered and singular genes involved in secondary metabolism, many of which are expressed during plant infection and may play a role in pathogenicity or niche competition. However, the enzymatic products of a major proportion of these genes remain undetermined. We report on-going efforts in the detection, isolation and full structure characterization of an unknown secondary metabolite from F. graminearum. The unknown compound is produced by numerous F. graminearum strains cultured in liquid media. Selective gene disruption and add-back experiments have identified the gene responsible for the biosynthesis of the unknown. A combination of HRESI/MS, 1D and 2D-NMR experiments were employed to elucidate its structure. The results of these combined experiments as well as a potential structure for the unknown compound is proposed.
**Fusarium** interspecies interactions during infection of durum wheat

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*Fusarium* cereal pathogens are known to produce a diverse array of mycotoxins and other secondary metabolites which may act together to cause plant disease and adverse effects on human and animal health. To study the impact of *Fusarium* interactions, we chose *F. graminearum* and *F. avenaceum*, which are known to co-contaminate Canadian durum wheat samples resulting in the deposition of trichothecenes, enniatins, moniliformin, and other bioactive compounds. Two *F. graminearum* (one 15ADON- and one 3ADON-producer) and two *F. avenaceum* strains were inoculated individually and in combination in durum wheat spikes in growth chamber studies. Fungal biomass was monitored using species-specific droplet digital PCR assays and transcriptomes were profiled by RNASeq. We observed that co-inoculations of *F. graminearum* and *F. avenaceum* led to reduced disease and deoxynivalenol levels compared to single inoculations of *F. graminearum*, even though *F. graminearum* greatly out-competed *F. avenaceum* in fungal biomass. We also conducted transcriptomic profiling of *F. graminearum* grown in the presence of *F. avenaceum* culture filtrate in vitro. An *F. graminearum* ABC transporter gene was strongly induced in planta after *Fusarium* species co-inoculations and in vitro upon exposure to *F. avenaceum* culture filtrate or enniatin B1. We are investigating whether this transporter is contributing to the ability of *F. graminearum* to compete during interspecies interactions.

**New fumonisins from Aspergillus niger and A. welwitschia**

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Fumonisins (FBs) are a class of mycotoxins produced mainly by *Fusarium* sp. The FB series of fumonisins are by far the most studied due to their toxicity. Sequencing of *Aspergillus niger*’s genome allowed for the discovery of gene clusters similar to those found in *Fusarium*, which are responsible for fumonisin biosynthesis. In our survey of Canadian strains of *Aspergillus sp*. isolated from grapes, we have detected the known fumonisins FB2, FB4, and FB6, which differ by the number of hydroxyl groups on the main backbone of the fumonisin structure. Using a MS/MS product ion filtering method in negative ionization mode developed specifically for the detection of the tricarballylic ester (TCE) functionality found on fumonisins, we’ve detected a new series of non-aminated fumonisins. Large scale fermentation on grapes and purification of these fumonisins allowed for 1H and 13C NMR characterization of these unprecedented fumonisins, which show that the amine functional group on FBx is replaced by a ketone (FPyx) or a hydroxyl (FLax) group. Using a duckweed (*Lemna minor*) assay, we show that the FPyx and FLax toxins are much less phytotoxic compared to the FBx series. Thus, the toxicity of fumonisins can for the first time, be experimentally attributed mostly to the presence of the amine functional group. Finally, insight into the biosynthesis via a stable isotope labeling study of the new fumonisins will be presented.

**Genomics and epigenetics of toxin production in Fusarium**

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Epigenetics is defined by ‘a stably heritable phenotype resulting from change in a chromosome without alterations in the DNA sequence’. The primary objective was to determine if *Fusarium graminearum* utilizes DNA methylation to regulate gene expression. In particular, we were interested to know if epigenetics played a role in the regulation of biosynthesis of deoxynivalenol (DON) in this fungus. A whole genome bisulfite sequencing (WGBS) was used to determine the presence of methylation at the individual base level across two isolates, DAOM241165 and DAOM233423 and under two environmental conditions. We observed that in nutrient-rich condition, 2.25% of the genome was methylated in the 233423 strain and methylation dropped to 1.82% in the nutrient deprived conditions. In the 241165 strain, ~1.6% of the entire genome was methylated in nutrient-rich conditions, and the
degree of methylation did not change under nutrient deprived conditions. A methylation specific PCR (MSP) was used to verify methylation patterns observed in WGBS. The gene Tri6 was chosen for analysis. Thus far, several methylated CpG sites observed by WGBS were confirmed by MSP. Future work will involve characterization of CpG sites in Tri6 and in other genes of the Tri gene cluster by MSP. Finally, in order to assess the impact of DNA methylation on gene expression, a DNA C-5 Methytransferase enzyme, FGSG_10766 was identified through homology to Neurospora crassa and Magnaporthe oryzae. Through a targeted gene knock out, we will examine changes in methylation patterns of the Tri genes and the production of DON.

An integrated approach to patulin reduction in food

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Patulin is a mycotoxin produced by a number of fungal species in the genera Penicillium, Aspergillus and Byssochlamys, particularly P. expansum, which is a major source of patulin contamination in infected fruits and vegetables, especially rotten apples and processed apple products including juice, cider and sauce. Although the acute toxicity is low, patulin has been shown to cause a variety of adverse effects, including nausea and vomiting in humans and neurotoxic, immunotoxic, genotoxic, teratogenic and carcinogenic effects and premature death in animals. Because of this, many countries have started to regulate levels of patulin in food. The maximum permissible levels of patulin set by most regulatory agents are 50 μg/kg for apple beverages, 25 μg/kg for solid foods and 10 μg/kg for baby foods.

P. expansum is a soil borne fungi and ubiquitously found. This poses great risk of contamination at nearly all links of the food value chain. Apple beverages for example, are typically produced from dropped, culled and low grade apples. These apples are more likely infected by patulin producing fungi, which can further grow during postharvest storage even at low temperature and in modified atmosphere. While growth of these fungi does not always lead to increased patulin production, our data have shown that the level of patulin contamination does correlate to storage length. Patulin is heat and acid stable, therefore thermal processing of apple juice or cider for example will not reduce patulin contamination in the products. This poses a great challenge for mitigation of patulin contamination in food. From field production of fruits and vegetables to processed food products, many factors can affect the growth of the fungi and production of patulin. Agricultural practices such as organic vs. conventional production, processing methods such as thermal vs. non-thermal novel methods, decontamination methods such as physical/chemical vs. biological, and other factors can significantly affect the outcome of patulin contamination in a finished food product. For this reason, an integrated approach is therefore being developed in our recent research as part of the Emerging Mycotoxins initiative, to mitigate patulin contamination in food.

Ergot fungi: a brief review of taxonomy and a preliminary phylogenetic study of Canadian isolates of Claviceps

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The ergot fungi infect the pistils of blooming florets of grasses (Poaceae), rushes (Juncaceae), and sedges (Cyperaceae), invade and colonize the ovary, and eventually replace seeds with sclerotia, which contain ergot alkaloids toxic to animals and human. They belong to the genus Claviceps (Clavicipitaceae, Hypocreales; Anamorph formerly called Sphacelia), which presently includes approximately 66 legitimate species names. In addition, fifteen Sphacelia species, which have not been connected to sexual states, may be connected to or reclassified in Claviceps. Most were described by morphological characters: shape, size and color of sclerotia, stromata, and conidia; presence or absence of hyphae cluster surrounding the base of stipe; floating or sinking of sclerotia in water, etc. Alkaloid profiles have been used to characterize a few species. Because most species have restricted host ranges (species, genus or tribe specific), host specificity can also be a useful character in species delimitation. However, Claviceps purpurea, the main pathogen of rye, but also oats, barley, wheat and other pasture grasses, infects more than 400 grass species around the world. Cross infection experiments were implemented to identify races. RAPD (Random Amplified Polymorphic DNA) identified high levels of variation. The multi-gene phylogenetic approach has only recently been applied by
Pažoutová et al. [1] and recognized four cryptic species (C. arundinis, C. humidiphila, C. purpurea s.str., and C. spartina) using samples predominantly of Europe origin. We amplified some of those gene regions to a set of samples of Canadian origin from barley, rye, wheat, and pasture grasses and conducted phylogenetic analyses. Results revealed high levels of genetic variation within C. purpurea s.str. and some likely recently diverged lineages.

References

Ergot alkaloids in sclerotia, wheat, and durum on the Canadian prairies

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Harvest surveys indicate the occurrence and severity of ergot infection in wheat and durum on the Canadian prairies varies from year to year, but has increased over the last decade and a half. The presence of ergot alkaloids in grain is correlated with the presence of ergot sclerotia. This is the basis many jurisdictions use to manage ergot contamination in grain. Durum and wheat harvested in 2011, a year in which ergot infection was particularly widespread in western Canada, showed a strong linear relationship between the concentration of ergot alkaloids and the presence of ergot sclerotia in grain. Inoculation experiments demonstrated that the identity of Claviceps purpurea isolates, as well as the resistance of host plant lines, affects the concentrations of ergot alkaloids produced in sclerotia. Total ergot alkaloid concentrations in sclerotia from various plants inoculated with the same C. purpurea isolate differed; however, the resistance of host plant line did not appear to be consistent with ergot alkaloid content in sclerotia.
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Man vs. Nature: prevalence of mycotoxins and climate change

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Mycotoxins, fungi and climate effects are unquestionably linked for many agricultural commodities. The prevalence of mycotoxins in grains is associated with the occurrence of the causal organisms and related causal factors, such as temperature and moisture. The key areas of both the field and storage in the supply chain are vulnerable to fungal infestation and require corresponding quality management processes to prevent or reduce potential mycotoxin contamination of the crop. Recent discussions have identified the climate-induced challenges facing global grain production. Examples from the review on ‘DON occurrence in grains: a North American perspective’ [1] will be used to discuss the links, the current status of research and farming practices and the challenges ahead.

References

Avoiding the unavoidable: regional impact and human counter measures to control mycotoxins

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Mycotoxins have plagued mankind since the beginning of organized crop production. Since the early 1960s, a wealth of information has been produced about mycotoxins and mycotoxicoses. However, even after 50 years of extensive research, mycotoxin problems still impact on public health and nutrition, income and sustainable development of much of the developing world. The major mycotoxins of agricultural importance on a global scale are aflatoxins, ochratoxins, fumonisins, deoxynivalenol/nivalenol, and zearalenone. This presentation highlights mycotoxin risks focusing on occurrence in foods and human exposure and influencing factors as well implications to agriculture, health and trade. As natural toxicants mycotoxins cannot be eliminated from the food supply but they can be effectively managed. Using aflatoxins, which are the only mycotoxins occurring in a wide range of produce both pre- and post-harvest, a holistic approach for effective management of mycotoxin problems is described. Aflatoxins are a major developmental challenge to Africa. The experience in using locally-relevant evidence and coordinated approach in aflatoxin mitigation in Africa in the past few years offers a model for generation of awareness and buy-in toward addressing complex problems.

Global regulatory interventions in the war against mycotoxins

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The U.S. Food and Drug Administration (FDA) enforces the Federal Food, Drug, and Cosmetic Act (FFDCA), a law intended among other things, to ensure that the U.S. food supply is safe for human consumption particularly when it may contain poisonous or deleterious substances such as mycotoxins. The FFDCA provides FDA the authority for regulating poisonous or deleterious substances, such as mycotoxins, in foods and to prohibit the entry of such foods into interstate commerce. For regulatory control of mycotoxins in foods, FDA monitors susceptible commodities for various mycotoxins, conducts science-based risk analysis, establishes regulatory limits, takes appropriate enforcement actions when warranted, and provides guidance to the food industry. FDA also works with other federal and state agencies on food safety issues related to mycotoxins, and participates in the Codex Alimentarius Commission, which implements the Joint Food and Agriculture Organization/World Health Organization Food Standards Programme to protect the consumer and promote fair practices in food trade.
Impact of mycotoxin regulations on world food trade

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When two nations engage in food trade (e.g., one nation exports food to the other), the food safety regulations of the importing nation determine whether the exporters’ commodity will be accepted. For example, when one importing nation has an aflatoxin standard of 20 μg/kg, it will not accept a lot of corn from another nation that has an average aflatoxin concentration of 30 μg/kg. However, it turns out that nations’ food safety regulations do not just affect the two trading nations in question, but trade patterns that have ripple effects across the entire world. Through our use of social network models to model the global food trade of corn and pistachios – two aflatoxin-contaminated commodities – we observe that ‘like attracts like’. The nations that have identical or near-identical aflatoxin standards trade substantially more food with each other than nations that have very different standards. This results in certain health risks accruing to populations in nations with relaxed or non-existent aflatoxin standards, and food security risks for nations that conduct food trade with relatively few other nations.

Impact of regulatory mycotoxin mitigation efforts

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Mycotoxins continue to be amongst the leading contaminants of interest to public health officials, regulators and food producers. Because of their natural occurrence in a variety of food products, many of which can be considered as staple foods, their management tend to be challenging. The serious adverse effects of toxins such as aflatoxins, ochratoxins, fumonisins, deoxynivalenol and zearalenone are such that the concern over their public health implications has increased over the years, leading several food regulators to take action to support reduction of human exposure to these substances in food. This paper will review some of the regulatory measures taken to support the control of a specific mycotoxin: ochratoxin A. It will assess adherence of these measures to a risk analysis based approach. Emphasis will be made on reviewing those considerations that go beyond the scientific outcomes of the risk assessment and which may have guided the decision-making process. To the extent possible, the estimation of the costs/benefits of these regulatory measures will be discussed.

Introduction of proactive risk-based decision making to mitigate mycotoxin risk

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While humans have always been exposed to mycotoxins through their diet, only recently have food producers, risk assessment agencies and regulatory bodies begun to implement sophisticated methods directed towards reducing consumer exposure to these naturally-occurring compounds. Regulatory agencies have set limits or action levels; and multiple agencies, both governmental and non-governmental, have created guidance on best practices for reducing contamination of food. The objective of these efforts is to reduce consumer risk, which is influenced by both the inherent hazard (toxicity) of these compounds and the amount of exposure (dose). Risk reduction is almost exclusively attained by reducing exposure, because of the difficulty in reducing the inherent toxicity of these chemicals. Since a reduction in exposure does not always correlate to a reduction in risk, it is important to consider whether the magnitude of that reduction will result in a significant reduction in risk determining, and not only whether mitigation efforts will reduce mycotoxin concentrations.

In 2015, the International Life Sciences Institute North America (ILSI North America) held a workshop to create a proactive, risk-based approach to assessing whether mitigation efforts are warranted for chemical compounds created during food manufacturing (process-formed compounds). The output of the workshop was a decision tree that uses scientific, objective criteria to predict whether mitigation efforts are likely to result in reduction in the risk to human health. The decision tree describes a five-stage process: (1) prioritization; (2) assessment of current risk; (3) development of mitigation plans; (4) evaluation of secondary effects of mitigation; and (5) recommendations. Within each stage, guidance is provided that drives the decision-making process based on risk. Participants at this workshop, which included representation from industry, academia and government sectors, identified that this approach
had value for food safety topics in addition to process-formed compounds, especially for other not-readily avoidable compounds such as mycotoxins. Mitigation programs continue to be developed for both mycotoxins currently identified as high priority (such as aflatoxins and deoxynivalenol), and for ‘new’ mycotoxins (such as sterigmatocystin and diacetoxyscirpenol) that have always been part of the food supply but are just now becoming the focus of food safety efforts. Maximizing resources dedicated to managing mycotoxins, in order to reduce consumer risk as much as possible, will require a proactive approach to evaluating mitigation efforts. The decision-tree developed by ILSI is one approach that could be used to ensure the maximization of human health benefits of mycotoxin mitigation efforts.

Use of state-of-the-art exposure assessment for mycotoxins

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Exposure assessment is a key step in determining consumer risk from mycotoxins, as risk cannot be determined from hazard alone. Accurate estimates of consumer exposure to mycotoxins also have important consequences for risk mitigation. While the hazard characterization of different substances can be updated and refined with new data and studies, hazard is nonetheless an intrinsic aspect of the substance itself and so in principle cannot be changed. Exposure on the other hand, is the combined result of mycotoxin occurrence and food intake, both of which can be to some degree be influenced by risk management decisions. Determining consumer exposure to mycotoxins requires having detailed data on their chemical occurrence in relevant foods, knowledge of how those foods are consumed by different populations, and estimates of the inherent variability associated with each input. Chemical occurrence data for mycotoxins can have a number of considerations, such as the frequency of occurrence, the level of concentration, the environmental or processing factors influencing these, and the limits of detection of the analytical equipment used to determine occurrence and concentration. Food consumption is generally accounted for using national food consumption surveys, which detail food intakes over a number of 24-hour periods for a statistically representative sample of consumers. These two inputs are then combined to give a distribution of exposure to a given mycotoxin in the population.

A number of different methodologies exist for characterizing dietary exposure in a population of consumers, and these methods vary in different regions. These range from screening level type calculations based on summary statistics to more sophisticated techniques that include probabilistic modelling, Monte Carlo simulations, and other statistical techniques that can be used to estimate the distribution of exposure to a mycotoxin in a population. Other considerations that need to be taken into account are how to compare exposure with a health-based guidance value, how to pick an appropriate point in an exposure distribution to determine risk, and an assessment of the uncertainties inherent in a given exposure assessment. These considerations and others will be discussed, with particular focus on their relevance for the exposure assessment of mycotoxins.

Mycotoxin mitigation as a model for risk-based decision making

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Risk-based decisions are those in which the need to achieve a significant level of risk reduction is the predominant guiding factor. Factors such as technical achievability, offsetting health benefits, and costs may also affect some decisions, but generally health protection is the primary consideration. The need to pursue risk reduction arises when some agent present in food (or any other medium of human exposure) is shown to pose a risk to health that is, by some measure, considered to be excessive. The priorities for risk reduction are influenced by the magnitude and nature of existing risk, the certainty with which it is known, and the requirements of law and regulation. Legal and regulatory requirements for risk reduction are most clear when the risk issue pertains to intentionally introduced food substances (direct and indirect additives, pesticide and veterinary drug residues); in such cases, substances cannot be introduced into food unless human intakes are less than defined risk levels, represented by ADIs. Note that methods currently used to derive ADIs do not allow identification of the quantitative magnitude of risk at, above, or below the ADI; the ADI is characterized in purely qualitative terms (‘reasonable certainty of no harm’). Thus, although the size of the risk at exposures in excess of, or even below, the ADI is not specified, there is general recognition that only intakes below the ADI can be allowed for intentionally introduced substances.
Substances, such as mycotoxins and other common food contaminants, present much greater challenges to decision-making than do intentionally introduced substances. Although data may exist to allow derivation of Tolerable Daily Intakes (TDIs) for such substances, decisions that simply declare that levels in food are excessively risky because they create human intakes in excess of applicable TDIs, and that only levels below a specified tolerance are legally permitted, do little to mitigate overall population risk. Unlike intentionally introduced substances, the levels of mycotoxins that come to be present and remain in affected foods are highly variable and not under human control. Regulatory decisions that simply result in monitoring of foods to detect violations of enforceable tolerances cannot mitigate more than a small fraction of the problem. Effective mitigation of mycotoxin risks should be based on modern concepts of risk-based decisions for complex environmental matters (see the 2008 National Research Council report, Science and Decisions). Once the public health problem associated with existing conditions is well-defined, risk assessments are conducted to evaluate the degree of risk reduction achieved with different technical or regulatory interventions that are viable options for risk mitigation. Attention is also given to any increased risks that may arise because of an intervention effort, something that is frequently possible but often ignored. Uncertainties in the risk assessments are fully described in forms useful for decision makers. Decisions (risk management) then focus on those interventions that provide greatest net benefit. The role of economic costs in those decisions is a matter of applicable regulatory policies.

Finally, it should be emphasized that measures of risk, risk reduction, and uncertainty are necessary under this modern decision framework. The use of ADIs or TDIs, which are ‘bright line’ models without associated risk measures, and with poorly described uncertainties, are not highly useful. Margin-of-Exposure (MOE) approaches, although also not truly risk measures, are nevertheless more useful, because there is some relationship between increases in MOEs and decreases in risk. Efforts to quantify those decreases are becoming available. Current models for quantifying carcinogen risk, which are applicable to several important mycotoxins, are well suited for risk-based decision making.
Aflatoxin biosynthesis and the related metabolism(s)

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Aflatoxins are carcinogenic and toxicogenic secondary metabolites produced mainly by Aspergillus flavus and A. parasiticus. The contamination of food and feed crops with aflatoxins has serious effects on the health of animals and humans. Aflatoxin biosynthesis is a multistep process composed of more than 25 enzymatic reactions, and the related genes belong to a 70-kb gene cluster. Although all genes in the aflatoxin gene cluster are essential for aflatoxin biosynthesis, the vrdA, which is not in the gene cluster, also participates in aflatoxin biosynthesis to make a metabolic grid in the pathway. It is well known that aflatoxin production by fungi is remarkably affected by nutritional and environmental factors such as carbon sources and temperature in culture conditions. Omics analyses are useful tools for characterization of bioprocesses and for search for novel strategies to control a target metabolism. We herein did metabolomics analyses using capillary electrophoresis mass spectrometry (CE-MS) to investigate the effects of carbon source and temperature on a wide range of metabolism of A. parasiticus. Our preliminary data showed that there were remarkable quantitative differences in some metabolites depending on the culture conditions. These results suggested that metabolomics analysis is a promising tool to clarify regulation mechanism of aflatoxin biosynthesis.

Aspergillus flavus secondary metabolic gene clusters: the good, the bad, and the bashful

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Aspergillus flavus is known mainly for its ability to produce toxic and carcinogenic aflatoxins. However, in silico analysis of its genome sequence has shown that it has the potential to produce numerous other secondary metabolic gene clusters; and in only a subset of these has the potential biological functions of the metabolite been determined. With so few metabolites identified for the predicted 56 SM gene clusters, it is important to identify as many of these compounds as possible to determine their bioactivity with respect to fungal development, survival, virulence and especially with respect to any potential synergistic toxic effects with aflatoxins. To better illustrate why these types of studies will provide novel information on the roles that SMs play in A. flavus pathobiology, recent studies on uncharacterized SM gene clusters have revealed the following metabolites: (1) a polyketide-derived pigment found exclusively in sclerotia was shown to impart UV and heat resistance to sclerotia and present antifungal properties, suggesting a role in fungal survival and (2) a polyketide-derived sclerotial metabolite, aflavarin (cluster 39), demonstrating antifungal properties was also shown to play a role in sclerotial production. While the asparasone and aflavarin clusters were expressed under laboratory conditions analysis of RNA-Seq data from a number of A. flavus strains growing on artificial media indicates that approximately half of the SM gene clusters are silent or expressed at very low levels. One method to induce expression of silent gene clusters is to overexpress the pathway-specific transcriptional activator gene, if present. Work in our laboratory showed that overexpression of the pathway-specific transcriptional activator in cluster 23, coupled with comparative metabolomics enabled identification of (3) a family of hybrid PKS-NRPS-derived 2-pyridones, designated leporins, never before described in A. flavus involved in development and possibly in iron homeostasis. Analysis of uncharacterized SM gene clusters in A. flavus will provide an excellent opportunity to study the genetics, biochemistry and ecological roles of these clusters that in turn will help us to predict the conditions that favor production of their associated metabolites in food and feed crops. If any of these metabolites are found to play a role in fungal development, virulence or
pathogenicity, they could serve as targets for disease control and reduction of aflatoxin contamination by *A. flavus*.

**Ochratoxin A biosynthetic pathway and its regulatory mechanisms**

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Ochratoxin A (OTA), mainly produced by different *Aspergillus* and *Penicillium* species, is one of the most important mycotoxin contaminants in agricultural products. It is known for its nephrotoxicity, hepatotoxicity, carcinogenicity, teratogenicity, and immunosuppression in humans and animals. OTA structurally consists of a dihydrocoumarin moiety linked with L-phenylalanine via an amide bond. OTA biosynthesis has been putatively hypothesized, although several different views exist on some processes of the biosynthetic pathway. We have sequenced the whole genome of *Aspergillus ochraceus* fc-1 strain and performed a comparative sequence analysis of the *A. ochraceus* fc-1 strain and other *Aspergillus* species. We could identify two new OTA-related polyketide synthase (PKS) (AoOTApks) genes. The predicted amino acid sequence of AoOTApks-1 displayed high similarity to previously identified PKSs from OTA producing *A. carbonarius* ITEM 5010 (67%; [PI] no. 173482) and *A. niger* CBS513.88 (62%; XP_001397313). However, the predicted amino acid sequence of AoOTApks-2 displayed lower homology with *A. niger* CBS513.88 (38%) and *A. carbonarius* ITEM 5010 (28%). A phylogenetic study on the β-ketosynthase and acyl-transferase domains of the AoOTApks proteins showed that they are sharing a common origin with other OTA-producing species, such as *A. carbonarius*, *A. niger*, and *A. westerdijkiae*. Also, the expression of AoOTApks-1 and -2 was positively correlated with the OTA concentration. ∆AoOTApks-1 and ∆AoOTApks-2 produced nil and lesser OTA, respectively than the wild-type strain. Our study suggests that AoOTApks-1 could be involved in OTA biosynthesis while AoOTApks-2 may be performed for an involvement in OTA production indirectly. Based on our whole genome sequencing analysis, we have identified other genes involved in the OTA biosynthesis and different regulatory elements that control the biosynthesis of OTA.

**Lipid-mediated signaling between fungi and plants govern production of mycotoxins**

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Contamination of seed with aflatoxin and fumonisin is a serious food and feed safety hazard as these mycotoxins are the most potent natural carcinogens that adversely affect diverse farm animals, poultry and humans. They are produced upon infection of corn ears by the seed-infecting fungi *Aspergillus flavus* and *Fusarium verticillioides*. Plant and fungal oxylipins are oxygenated fatty acids produced by diverse oxygenases including lipoxygenases (LOX). Plant oxylipins function as signals in defense and development. In fungi, oxylipins are potent regulators of mycotoxin biosynthesis and sporogenesis. Recent evidence suggest that plant and fungal oxylipins may act as signals in cross-kingdom communication that either induce or inhibit disease progression and pathogenesis. To test this hypothesis, corn and *A. flavus* oxylipin-deficient mutants were created and tested for any alterations in the ability of the fungus to colonize seed and produce spores and the mycotoxin aflatoxin. Disruption of specific LOX genes resulted in either decreased or increased resistance to leaf blights, stalk rots and to contamination with fumonisins and/or aflatoxins with the outcome of interactions dependent on specific LOX genes and specific pathogens. The results will be presented showing that the host and fungal oxylipin metabolism governs the outcome of corn-fungal interactions and that production of plant oxylipins may be modulated by fungi for their own advantage to facilitate pathogenesis and production of spores and mycotoxins.
Breeding corn for resistance to *Fusarium* ear rot: an integrated approach of transcriptomics, metabolomics and genomics

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*Fusarium verticillioides* is a fungal corn pathogen that causes ear rot and contaminates the grains with fumonisin mycotoxins. Genomic regions and candidate genes for kernel resistance to *F. verticillioides* infection were detected through the comparison of resistant and susceptible corn inbred lines, by adopting three different approaches: transcriptomic (RNASeq), metabolomics analyses and Quantitative Trait Locus (QTL) mapping. Comparison of expression levels between inoculated vs. uninoculated and resistant vs. susceptible transcriptomes revealed a total number of 6,951 differentially expressed genes. Differences in constitutive gene expression were observed in the uninoculated samples. The resistant genotype showed a higher level of expression of genes distributed over all functional classes, in particular those related to secondary metabolism category. After *F. verticillioides* inoculation, a similar response was observed in both genotypes, although the magnitude of induction was much greater in the resistant genotype, including numerous transcripts encoding components of signal transduction cascades and enzymes required for the synthesis of secondary metabolites.

The same kernel samples were subjected to a metabolomics analysis by liquid chromatography in combination with high resolution mass spectrometry. The use of stable isotopic labelling combined to MetExtract algorithm allowed the automatic detection and prediction of carbon atoms of only compounds of biological origin. The most intense ion species was selected for each feature group and 534 feature groups, corresponding to likewise metabolites, were issued. Several databases were screened for the metabolite search of the highest m/z within each feature group restricted by the count of the carbon atoms. A total of 222 metabolites resulted significantly affected by *F. verticillioides* inoculation. Annotation revealed that a large amount was involved in the synthesis of oxylipins, phenylpropanoids, flavonoids, benzoxizanoids, hydroxycinnamic acid amides and aromatic amino acids.

A segregating F2:3 population of 188 progenies was developed crossing resistant and susceptible genotypes. *Fusarium* ear rot (FER) severity and fumonisin B1 contamination content were evaluated over two years and two sowing dates (early and late) on ears artificially inoculated with *F. verticillioides* by the use of either side-needle or toothpick inoculation techniques. Significant positive correlations ($p<0.01$) were detected between FER and FB1 contamination, ranging from 0.72 to 0.81. A genetic map was generated for the cross, based on 72 microsatellite markers and 341 single nucleotide polymorphisms derived from Genotyping-by-Sequencing. QTL analyses revealed six QTLs for FER, eight QTLs for FB1 contamination and three of them were in common between the two traits, located on linkage group 2, 7 and 9. Finally, 25 candidate genes for resistance to *F. verticillioides* were identified combining transcriptomic data with QTL mapping, providing a set of genes that could be further studied to evaluate their usefulness in marker assisted selection.
Mycotoxin binders for the reduction of aflatoxins in animals and humans

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Global concerns about the safety of food have evoked a growing awareness of the hazards associated with aflatoxins. Appropriate interventions that can mitigate toxin exposures in the vulnerable are high priorities for both humans and animals. In vitro and in vivo studies were designed to test the hypothesis that the inclusion of a dioctahedral smectite clay (NS, UPSN, ACCS100) in the diets of animals and humans can be used as an effective strategy for decreasing exposures and aflatoxicoses. In mechanistic studies, we demonstrated that the intact dicarbonyl system in aflatoxin is essential for optimal sorption by clay. Thermodynamic evidence also suggested that aflatoxin reacts at multiple sites on clay surfaces. In vivo studies have shown that UPSN clay tightly and preferentially sorbed aflatoxins in the gastrointestinal tract resulting in decreased aflatoxin bioavailability. Clay intervention trials in communities from Texas, the northern Ashanti region of Ghana, and the Eastern Province of Kenya demonstrated significant reductions in biomarkers of aflatoxin exposure in urine and blood and suggest that clay treatment is well-tolerated by participants, including children. Moreover, dose delivery (capsule, food, or flavored water) has varied by study suggesting delivery can be modified for the population at risk. Clay-based enterosorption of aflatoxins is a practical, culturally acceptable and sustainable approach that will benefit more than 4.5 billion people living in climates conducive to the growth of fungi and the production of mycotoxins in staple foods.

Recent advances in microbial and enzymatic detoxification of mycotoxins – epimerization of deoxynivalenol

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Microbial and enzymatic detoxifications represent very promising alternatives for addressing the ever growing dilemma of mycotoxin contamination in food and feed commodities. While breakthrough findings supported by genuine industrial interests led to the optimization of limited enzymatic solutions (such as FUMzyme®), other mycotoxins such as deoxynivalenol (DON) are still awaiting for more robust innovative approaches. Notwithstanding the fact of being the most widely detected mycotoxin worldwide, the biochemical nature of DON makes the pursuit of potential detoxifying-bacteria/enzymes more challenging from the standpoint of view. Several aerobic and anaerobic microorganisms selected from ruminants, swine, poultry, fish, and other agricultural commodities have been found to exhibit various DON transformation capabilities [1-7]. The need for restrictive conditions during DON bio-transformation such as an anaerobic atmosphere [2,3] and/or the presence of DON as the sole carbon source [6,7] was among the greatest challenges that faced in developing practical applications of these isolates. The most recent isolation and characterization of Devosia mutans 17-2-E-8, a novel gram-negative bacterium capable of transforming DON to a non-toxic epimer, 3-epi-DON, under aerobic conditions is opening doors for better management strategies of this mycotoxin.

The lately obtained data indicates that the enzymatic epimerization process proceeds through a two-step bio-catalysis. DON is initially oxidized to a short living intermediate, 3-keto-DON, that is specifically reduced in a later stage to 3-epi-DON. The reaction kinetics indicated that 3-epi-DON is the stable end product of this reaction nonetheless some earlier reports that suggested 3-epi-DON as an intermediate metabolite in similar bio-catalysis systems found mainly in gram-positive bacteria (such as Nocardiooides sp. Strain WSN05-2). The noticeable high ratio of 3-epi-DON to 3-keto-DON suggests an enzymatic reaction that favors the formation of the stable isomer, 3-epi-DON. This proposes an irreversible bioconversion system of DON which particularly addresses many of the issues observed earlier in connection with the exploitation of enzymatic biotransformations for detoxification purposes, e.g., DON acetylation by Tri101 acetyl transferases. Further studies are underway to confirm the function of the identified enzymatic systems and optimize them for more sustainable agricultural approaches to mitigate Fusarium toxin contaminations. Considering the involvement of DON in the pathogenesis of different Fusarium species, it looks very plausible to develop plants that are resistant/tolerant Fusarium diseases by utilizing the epimerization process of DON. The aforementioned enzymes would also have the
capacity to minimize the possible passage of DON contamination to food/feed value chains when applied in post-harvest treatments such as the wet-milling of grains or semi-liquid feeding of animals.

References

Novel approaches to assess mycotoxin detoxification methods in feed – biomarkers of exposure and effect

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Although in vitro experiments are crucial tools in the development of mycotoxin deactivators, in vivo studies are mandatory for investigating the efficacy of mycotoxin inactivation agents in animals. The best-suited indicators for assessing mycotoxin exposure and the extent of mycotoxin inactivation are mycotoxin biomarkers. This presentation will focus on the selection of biomarkers and on the application of different biomarker methods for investigating the inactivation of the Fusarium mycotoxins zearalenone (ZEN) and fumonisins B1+B2 (FB1+FB2) in pigs.

ZEN is readily absorbed from the GI tract and in part reduced to α-zearalenol (α-ZEL). Both ZEN and α-ZEL are glucuronidated to great extent and excreted via urine and via bile. The biomarker of choice for evaluating the efficacy of feed additives for inactivation of ZEN is the molar sum concentration of ZEN and its metabolites in urine after normalization to creatinine. This approach was applied a) to demonstrate the in vivo inefficacy of two lignocellulose binders that had previously shown in vitro adsorption values of ≥ 90% and b) to prove the ability of a lactone hydrolase to cleave ZEN to its non-estrogenic metabolite hydrolyzed ZEN (HZEN) in the GI tract of pigs. In this presentation, both animal trials will be discussed and the challenges associated with biomarker analysis for binding and enzymatic conversion as mycotoxin inactivation strategies will be pointed out.

Unlike ZEN, fumonisins are poorly absorbed from the GI tract of pigs. Hence, determination of FB1, FB2 and their metabolites in urine demands very sensitive analytical methods. However, if feed additives based on enzymatic or microbial conversion of fumonisins are evaluated, determination of the fumonisin metabolite pattern in feces is an easy alternative to urine analysis. Yet, as the fumonisin pattern in feces does not exactly reflect the fumonisin pattern at the time point of absorption, the sphinganine to sphingosine (Sa/So) ratio in serum, a biomarker for long-term exposure to fumonisins, is usually monitored, too. Combination of the fumonisin pattern in feces as biomarker of exposure and the Sa/So ratio as biomarker of effect was used in several pig experiments to demonstrate the efficacy of the commercially available feed additive FUMzyme to cleave FB1 and FB2 to their much less toxic hydrolyzed metabolites in vivo. This presentation will summarize some of these trials and point out difficulties that might arise when feed additives for inactivation of fumonisins are tested at the guidance value of 5 mg/kg FB1+FB2 in pig feed.
Round table discussion: Current and future legislation of mycotoxin binding and detoxifying products

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FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures) is the united voice of the specialty feed ingredients business in Europe. Our membership comprises manufacturers and traders of feed additives, functional feed ingredients, premixes and other mixtures of specialty ingredients that enter the food chain via feed. FEFANA facilitates the dialogue between EU institutions and feed business operators while promoting feed and food safety and a fair and competitive market. For any additional information, please contact the FEFANA Secretariat at eeig.additives@fefana.org or visit our website (www.fefana.org).

Mycotoxin deactivators are a relatively new category of feed additives that comprise a very heterogeneous group, from substances adsorbing mycotoxins (such as clay minerals or yeast derivatives) to those that biotransform them (e.g., microbial strains or enzyme preparations). Until today, only a few feed legislations allow the authorization (and therefore, the marketing) of mycotoxin deactivators as such. Some authorities regulate these products according to a principal characterization apart from their mycotoxin-deactivating claims. This is the case for clay minerals (with mycotoxin-adsorbing claims) that are often authorized as anticaking-agents only – because a mycotoxin-specific authorization is not foreseen in the respective legislation. In the European Union (EU), there was no regulatory framework for the authorization of mycotoxin deactivators until 2009. Therefore, products having only a mycotoxin-deactivating claim were effectively banned from the market until then. The situation changed with the publication of Commission Regulation (EC) No 386/2009 establishing a new functional group of feed additives: substances for reduction of the contamination of feed by mycotoxins. However, even with this regulation in place, only three such products have been authorized in the EU to date. One reason might be that the corresponding guidelines (Commission Regulation EC No 429/2003) on how to compile a registration application dossier has not been provided or updated accordingly. From an industry point of view, a clear regulatory framework on a national (or even supranational) level for the authorization of mycotoxin deactivators is desirable. Such a regulatory framework should set defined rules and requirements for the authorization process and would, as a consequence, encourage only effective products to be placed on the market. FEFANA was and is participating in the process of defining such rules and requirements in the EU.

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The EU has since many years extensive regulations on mycotoxins in feed and food. Prevention is better than cure to protect the humans and animals from the toxic effect of mycotoxins. However, in recent years, an increased prevalence and a significant year-to-year variation of the presence of mycotoxins in feed and food in the European region can be observed. Major cause is climate change and in particular the extreme weather conditions during critical growth stages of cereals in particular corn. The high levels of aflatoxin in the corn harvest 2012 and the high level of Fusarium toxins in the corn harvest 2013 and 2014 have resulted in problems for feed and food supply and safety. This situation entails specific challenges for farmers, feed and food manufacturers, traders and regulators to ensure the safety for animal and human health of feed and food while ensuring the supply of major staple feed and food such as cereals.

Regulation (EC) No 386/2009 has established a new functional group of feed additives in the category of technological additives: ‘substances for the reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action’. Since the creation of this new functional group, several mycotoxin binding and detoxifying products have been authorized at EU level. To which extent shall mycotoxin binding and detoxifying products contribute to a sustainable solution for the mycotoxin challenge?
Approval process for substances for use in animal foods to eliminate, bind, or transform mycotoxins.

The adverse effects of mycotoxins on animals and humans have led to increasing interest in the use of substances such as immune-stimulators, clays, and enzymes in animal food to eliminate these adverse effects. Substances intended to be immune-stimulators can affect the structure and function of the animal and may be considered new animal drugs (NADs), which are required to go through the New Animal Drug Approval process to demonstrate safety and effectiveness. Clays, such as bentonite and hydrated sodium calcium aluminum silicates, are potentially being added to animal food with the intent to bind mycotoxins in animal foods. Enzymes and chemicals (such as ammonia, ozone, and sodium hydroxide) are potentially being added to animal food for the sole intent of transforming the mycotoxins to non-toxic substances in the food. This binding or transformation of mycotoxins in animal food is considered an effect on the characteristics of the food itself. Under section 201(s) of the U.S. Food Drug and Cosmetic Act (21 U.S.C. § 321(s)), substances that may be expected to become a component of the food or affect the characteristics of the food itself are considered food additives, except for substances that are generally recognized as safe (GRAS) for an intended use; pesticides; color additives; or drugs. Section 409 of the Food Drug and Cosmetic Act (21 U.S.C. § 348) requires the approval of the food additive by the FDA before it can be used in animal food. Approval of a food additive is accomplished through the Food Additive Petition (FAP) process, which is described in Title 21 of the Code of Federal Regulations part 571 (21 CFR Part 571). Within FDA, the Center for Veterinary Medicine (CVM) is responsible for the review and approval of animal FAP. When FDA approves a FAP, a regulation is established in 21 CFR Part 573 addressing the safe use of the food additive in animal food. In June 2015, FDA published Guidance for Industry #221 on the Recommendations for the Preparation and Submission of Animal Food Additive Petitions. This guidance describes the types of information CVM recommends for inclusion in FAP for food additives used in animal food, including: the name and all pertinent information concerning the food additive; chemical identity and composition of the additive or manufacturing methods and controls; intended use, use level and labeling; data establishing the intended effect (physical, nutritional, or other technical effect); a description of analytical methods to determine the amount of the food additive in the food; safety evaluations for the animal and humans consuming products from those animals; proposed tolerances for the food additive; proposed regulation; and environmental assessment. The goal of the guidance is to help the animal industry understand what information they need to submit in a FAP to establish the safe use of the substance and help ensure the safety of the human and animal food supply.

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No regulation on mycotoxin binders/deactivators as feed additives has entered into force in China. As such, no standard protocol is available to verify the efficacy and safety of mycotoxin binders/deactivators on the Chinese market. In 2013, the Ministry of Agriculture initiated a 5-year project entitled 'Mycotoxin detoxifying feed additives standard drafting' that includes the following main objectives.

2. A survey on regulation of mycotoxin deactivators and their application in the market from major countries.
3. A survey on heavy metal content in mycotoxin deactivator products.
4. A survey on methodologies for evaluation of efficacy for mycotoxin deactivators.
   4.1. Collecting the in vitro and in vivo evaluation methodologies from major countries and testing some representative deactivators from the Chinese market.
   4.2. Nutrient binding test for mycotoxin deactivators.
   4.3. Set up a standard in vitro methodology for mycotoxin deactivator evaluation according to 4.1 and the local market condition.
   4.4. Set up a standard in vivo methodology for mycotoxin deactivator evaluation according to 4.1 and the local market condition.
5. Set up the 'Mycotoxin detoxifying feed additives standard drafting' according to 4.
   5.1. In vitro methodology.
   5.2. In vivo methodology.
   5.3. A guideline for efficacy evaluation will be launched by the Ministry of Agriculture by December 2016.
THURSDAY, JUNE 9, 2016 – Plenary meeting

After 125 years of mycotoxin research, what is expected from researchers now?

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I believe that there are three important societal expectations of mycotoxin researchers in the fully developed market economies. The first challenge that requires our attention is the influence of climate on the distribution of fungal toxins in crops and feeds. The next 10-20 years will see the need to create analytical capacity in geographic areas that had been used to stable patterns of contamination. That is to say old toxins in new places.

The best evidence is that the genetics of the populations of key toxigenic fungi are changing partly with climate change. The fungi that cause *Fusarium* head blight/*Gibberella* ear rot are changing. This is due in part to the movement of strains that make deoxynivalenol (DON) via 3ADON into regions hitherto occupied by 15ADON strains. There is clear evidence that these populations are recombining in unexpected ways. Maintaining a satisfactory margin of exposure below the TDI for DON is already a challenge in some regions. This will require more refined exposure assessments (biomonitoring) to protect vulnerable populations. The regions where *Aspergillus flavus* can thrive appear to be expanding due to warming. The discovery of the sexual state of *A. flavus* challenges the present understanding of these fungi. Sex within populations of the aflatoxigenic aspergilli may create additional difficulties for the control of aflatoxin in food and feed.

In the face of these challenges, it seems likely it will become more common to divert lower quality grains into feed. Warmer winters in cool season areas where silage is a common feed source will increase the occurrence of the toxins from the species mainly of *Penicillium* that grow in silage. Not much is known about the effects of these toxins on animal health and productivity and their distribution in animal products (especially milk). Thus my third major challenge for the decades ahead is to refine the tools used through the feed value chain to minimize the impact of a greater risk of lower quality feeds on animal health and productivity.

Finally, it must be said that in parts of Africa, Latin America and Asia exposures to aflatoxin and/or fumonisin are at levels that affect population health. This has been recognized for 50 and 20 years, respectively and if anything the problems are getting worse. Addressing this is perhaps one major expectation of all mycotoxin researchers now.
POSTERS

OCCURRENCE

P1 – P21

P1  The epidemiology of Fusarium langsethiae in oats
Heidi U. Aamot¹, I. Klingen¹, S G. Edwards², M. Brurberg¹, G. Brodal¹, T.S. Eklo¹, H.S. Steen¹, J. Razzaghian¹, E. Gauslå¹ and I.S. Hofgaard¹
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P2  Characterization of aflatoxigenic Aspergillus spp. and aflatoxin contamination of imported foods originated in Asia countries in Scotland
S. Ruadrew, J.A. Craft and Kofi E. Aidoo
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P3  Prevalence, mycotoxin production ability and characterization of Fusarium species on Korean adlay (Coidic lacrymal-jobi L.)
Tae Jin An¹, Y.G. Kim¹, S.W. Cha¹, Y.S. Moon³, S.H. Yu² and S.K. Oh²
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P4  Occurrence of Fusarium mycotoxins in Croatian cereals harvested in 2015
Lidija Brodar¹, T. Klapec¹, M. Sulyok², R. Krksa² and B. Šarkanj¹
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P5  Occurrence of aflatoxins in farming fish from São Paulo State, Brazil
M.M. Massocco¹, E.C. Michelin¹, S.H.S. Godoy¹, G.S. Yasui², G.E. Rottinghaus³, R.L.M. Sousa¹ and Andrezza M. Fernandes¹
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P6  Frequency and concentration of aflatoxins and fumonisins in corn in Kenya and Tanzania: implications for food safety
B. Temba¹,², D. Kriticos¹,³, J. Beddow⁴, R. Darnell³, G. Fox¹, A. Gichangi⁵, J. Harvey¹,⁶, J. Karanja³, F. Liebenberg⁷, D. Lwezaura⁸, S. Massomo⁹, R. Ngeno⁶, N. Ota³, P. Pardey³, J. Wainaina⁸, I. Wanjuki³ and Mary Fletcher¹
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P7  Assessment of mycotoxins in organically and conventionally grown crops in temperate regions
G. Brodal¹,², Ingerd Skow Hofgaard¹, G.S. Eriksen³,², A. Bernholt² and L. Sundheim¹,³
¹Norwegian Institute of Bioeconomy Research (NIBIO), ²Norwegian Veterinary Institute and ³Norwegian Scientific Committee for Food Safety, Norway

P8  The occurrence of mycotoxins in Polish silage – a preliminary study
Piotr Jedziniak¹, M. Piątkowska¹, O. Burek¹, K. Pietruszka¹, E. Kukier² and K. Kwiatek²
¹Department of Pharmacology and Toxicology and ²Department of Hygiene of Animal Feedingstuffs, National Veterinary Research Institute, Poland

P9  Detection of aflatoxin B1 contamination in imported chia seed dietary supplements in Thailand
Usuma Jermnak, A. Poapolathee, S. Poapolathee, K. Imsilp and P. Tanhan
Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Thailand
A global survey of regulated, masked and emerging mycotoxins and secondary metabolites in agricultural products from 2012-2015

**Gregor Kos**1, P. Kovalsky2, K. Nährer2, C. Schwab2, G. Schatzmayr2, M. Sulyok3 and R. Krska3
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Comparative analysis of fusariotoxins occurrence in different types of corn materials

**Julia Laurain**1, M.A. Rodriguez1, E. Nacer Khodja1 and E. Marengue2
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Comparative analysis of fusariotoxins occurrence in wheat, barley and corn grain

**Julia Laurain**1, M.A. Rodriguez1, M. Gallissot1 and E. Marengue2
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A UK total diet study of mycotoxins

Fera Science Ltd., UK

Comparative metabolism study of HT-2 and T-2 toxin in small grain cereals

**Jacqueline Meng-Reiterer**1, E. Varga1, A. Nathanail2, C. Büschl1, G. Adam3, F. Berthiller1, M. Lemmens4 and R. Schuhmacher1
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European survey on sterigmatocystin in cereals, cereals-based products, beer and nuts

**Hans G.J. Mol**1, S.J. MacDonald2, C. Anagnostopoulos3, M. Spanjer4 and A. Pietrie5
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Worldwide mycotoxin occurrence in feeds and raw materials – survey data 2015

**Michele Muccio**, C. Schwab, S. Masching, P. Kovalsky and K. Naehrer
BIOMIN Holding GmbH, Austria

The hidden threat – emerging mycotoxins

**Barbara Novak**, E. Mayer and G. Schatzmayr
BIOMIN Research Center, Austria

Epidemiology of Fusarium species producing enniatins in French wheat, durum wheat, barley and triticale

**Béatrice Orlando**1, K. Kasheifard1, G. Grignon1 and R. Valade2
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Co-occurrence of fusariotoxins naturally produced by Fusarium species in French corn

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Trichothecene mycotoxin levels detected in winter wheat in Ontario, Canada from 2009-2015

**Ljiljana Tamburic-Illinčic**
University of Guelph, Canada

Identification and characterization of Alternaria species causing early blight on potato in Belgium

**Michiel Vandecasteele**1, S. Landschoot1, K. Audenaert1, M. Höfte2, S. De Saeger3 and G. Haesaert1
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HUMAN AND ANIMAL HEALTH
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P22  *Is there a health risk concerning the children’s consumption of cereal-based products? A cumulative mycotoxin risk assessment approach*
R. Assunção¹,²,³, C. Martins¹,²,⁴, E. Vasco¹ and Paula Alvito¹,²
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P23  *Assessment of mycotoxins in human milk of celiac mothers*
C. Brera¹, F. Valitutti², A. Nigrì¹, C.M. Trovato², D. Iorfida² F. Debegnach¹, E. Gregori¹, M. Barbato², S. Cuccia², C. Catassi³ and Barbara De Santis¹
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P24  *Influence of cereal ergot on growth performance and digestibility in lambs*
Stephanie Coufal-Majewski¹,², T. McAllister¹, B. Blakley⁴, A.V. Chaves², Y. Wang¹ and K. Stanfors³
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P25  *Exposure to the mycotoxin zeralenone impairs embryo development in zebra fish*
N. Sidebotham, J. Duringer, R. Tanguay and A. Morrie Craig
OSU Endophyte Service Laboratory, USA

P26  *Experimental study of deoxynivalenol biomarkers in urine*
C. Brera¹, Barbara de Santis¹, Francesca Debegnach¹, B. Miano¹, G. Moretti¹, A. Lanzone², G. Del Sordo², D. Buonsenso², A. Chiaretti², L. Hardie³, K. White³, A.L. Brantsæter⁴, H. Knutsen⁴, G.S. Eriksen⁵, M. Sandvik⁵, L. Wells⁶, S. Allen⁶ and T. Sathyapalan⁶
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P27  *Carry-over of aflatoxin B1 from dairy cows’ feed to milk*
Ine van der Fels-Klerx and L. Camenzuli
RIKILT Wageningen UR, the Netherlands

P28  *Transfer of aflatoxin B1 from feed to lambari fish (Astyanax altiparanae)*
E.C. Michelin¹, M.M. Massocco¹, S.H.S. Godey¹, J.C. Baldin¹, G.S. Yasui², G.E. Rottinghaus³, R.L.M. Sousa¹ and Andrezza M. Fernandes¹
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P29  *PR toxin toxicity on human cells – an in vitro study*
M. Coton¹, O. Puel², H. Le Scouarnec†, E. Coton¹, Nolwenn Hymery¹
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P30  *Carry over of deoxynivalenol and its 3-sulfate metabolite into eggs of laying hens*
Elsa Kunz-Vekiri¹, N. Jurišić¹, H.E. Schwartz-Zimmermann¹, D. Schatzmayr², D.J. Caldwell³, J.T. Lee¹ and F. Berthiller¹
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P31 Stability of masked mycotoxins under gastrointestinal conditions in vitro
Susan MacDonald¹, J. Tarbin¹, J. Clough¹, I. Leon¹, C. Crews¹, S.W. Gratz², R. Dinesh² and G. Duncan²
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P32 Fumonisin B1 and ochratoxin A and their biomarkers determination in animal tissues and serum as an assessment of dietary exposure
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P33 Women and children exposure to mycotoxins: a case study of subsistence farmers in Shamva and Makoni Districts, Zimbabwe
Loveness K. Nyanga¹, T.C. Murashiki², D. Maringe¹, M. Dembedza¹, L.R. Manema¹, M.A. Benhura² and C. Chidewe²
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P34 The effect of aflatoxins in the diet of weaned piglets on health and nutrient digestibility
Anna-Katharina Oudshoorn and Y.M. Han
Trouw Nutrition Research & Development, the Netherlands

P35 Incidence of fusariotoxin co-exposure on human monocytes
Marie-Caroline Smith
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P36 Do cyclic depsipeptide mycotoxins beauvericin and enniatins cross the blood-brain barrier?
Lien Taevernier, N. Bracke, L. Veyser, E. Wynendaele and B. De Spiegeleer
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P37 Effects of Fusarium mycotoxins and feed restriction on broiler growth performance and immune response
Anhao Wang¹, T. Scott¹ and N. Hogan¹,²
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P38 Effects of feed-borne Fusarium mycotoxins on performance and intestinal histology in broiler chickens depends on timing of exposure
Anhao Wang¹ and N. Hogan¹,²
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P39 Aflatoxin exposure, gut function and child growth in Nepal
Sinead Watson¹, Y.-K. Tu², N. Mitchell², F. Wu³ and Y.Y. Gong¹
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SAMPLING & ANALYSIS
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P40 Determination of 25 mycotoxins in Western Canadian oats from 2014-2015 crop years using LC-ESI/MS/MS with fast polarity switching and scheduled MRMs
Richard Blagden, M. Roscoe and S.A Tittlemier
Grain Research Laboratory, Canadian Grain Commission, Canada

P41 The effect of grind size and extraction size on fumonisin result variability
C.K. Maune, C.L. Maune, J. Bierbaum, Julie Brunkhorst and R. Niemeijer
Trilogy Analytical Laboratory, USA
P42 A new look at citrinin: quantitative analysis by HPLC and LC-MS/MS utilizing immunoaffinity purification  
J. Bierbaum, C. Maune, Julie Brunkhorst and R. Niemeijer  
Trilogy Analytical Laboratory, USA

P43 The adsorption and desorption capabilities of an aluminum silicate analysis containing multiple mycotoxins  
A. Pope, Julie Brunkhorst, J. Bierbaum and R. Niemeijer  
Trilogy Analytical Laboratory, USA

P44 Development of a multiplex mycotoxin microarray  
S.E. McNamee¹, G. Rosar², F. Bravin², C.T Elliott¹ and Katrina Campbell¹  
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P45 Functions of a testing laboratory for mycotoxins and endophyte toxins  
A. Morrie Craig and L.L. Blythe  
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P46 Multimycotoxin LC-MS/MS screening in feed  
A. Biancardi¹ and Chiara Dall’Asta²  
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P47 Determination of ochratoxin A in meat, meat products and edible offal: preparing the intervalidation study for CEN standard method  
Barbara De Santis, C. Saitta, F. Debegnach, E. Gregori and C. Brera  
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P48 Sampling of grains stored in silos for mycotoxicological and nutritional evaluations  
A.O. Mallmann¹, J.K. Vidal¹, Paulo Dilkin¹, L.R.S. Wovst² and C.A. Mallmann¹  
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P49 Modeling uncertainty estimation for the determination of aflatoxins (B1, B2, G1, G2) in corn by liquid chromatography tandem mass spectrometry  
L.R.S. Wovst², Paulo Dilkin¹, A.O. Mallmann¹, M.S. Oliveira¹,² and C.A. Mallmann¹  
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P50 Validation of a method for the analysis of citrinin in cereals using immunoaffinity columns  
D. Leeman, E. Marley, C. Milligan and Carol Donnelly  
R-Biopharm Rhône, UK

P51 Multi-toxin analysis using immunoaffinity column clean-up for a range of samples prior to LC-MS/MS detection  
J. Wilcox, D. Leeman, E. Marley, C. Milligan and Carol Donnelly  
R-Biopharm Rhône, UK

P52 Determination of six major ergot alkaloids and their epimers in cereal grains using LC-MS/MS  
Dainna Drul, M. Roscoe and S.A. Tittlemier  
Grain Research Laboratory, Canadian Grain Commission, Canada

P53 Accurate lateral flow aflatoxin testing with environmental-friendly aqueous extraction  
Michael Fischer, V. Zimmermann, W. Lübbe and M. Mättner  
R-Biopharm AG, Germany

P54 Analysis for Fusarium mycotoxins in single kernels of wheat  
Don Gaba, J. Chan, W. Harnden, E. Thomas, J. Bamforth, M. Shahin and S.A. Tittlemier  
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Optimal sampling and extraction procedures for ergot measurements
Taylor Grusie1, V. Cowan2, W. Schwab3, B. Blakley1,2, J. McKinnon1 and J. Singh1
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The use of laboratory automation in the routine analysis of EU-regulated mycotoxins by UHPLC-MS/MS
Dan Hengst, T. Vennard and K. Mastovska
Covance Laboratories, USA

Evaluating the BGY fluorescence signal in corn kernels inoculated with various aflatoxin producing fungi using fluorescence hyperspectral imaging
Zuzana Hruska1, H. Yao1, R. Kincaid1, F. Zhu1, R. Brown2 and D. Bhatnagar2
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Approach to evaluate the impact of different structural moieties of ochratoxin A on its cytotoxicity by testing synthesized derivatives
U. Rottkord1, I. Ferse1, G. Schwerdt2, M. Gekle2 and Hans-Ulrich Humpf1
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Bioactive compounds from fungal cultures: identification, isolation and structure elucidation
B. Arndt1,2, S. Kalinina2 and Hans-Ulrich Humpf1,2
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Design of a novel microfluidics platform for mycotoxin food contaminant determination
Jonathan Loftus1, C. Loscher1 and R. O’Kennedy1,2
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High throughput automated cleanup and analysis of aflatoxin and ochratoxin
M. Pazdanska, R. Rhemrev, E. Marley, C. Donnelly and Elizabeth Manning
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Incorporation of recombinant Fab antibody fragments in a ‘point-of-site’, optical-planar waveguide biosensor device for detection of aflatoxin B1
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Development of method for the simultaneous determination of multi-mycotoxins in green coffee bean by LC-MS/MS after SPE cleanup
Ock Jin Paek, S.H. Kim, M.C Yoon, W.S. Nam, D.S. Kim and S. Suh
Food Contaminants Division, National Institute of Food & Drug Safety Evaluation, Ministry of Food and Drug Safety, Republic of Korea

Fit-for-purpose immunochemical test kits for fumonisins screening – different solutions for different needs
F. Gon, F. Diana, Maurizio Paleologo Oriundi and L. Persic
Tecna s.r.l., Italy

On-site study to evaluate rapid inspection of grain trucks on mycotoxins based on dust sampling
Mareike Reichel, T. Gronewold and S. Biselli
Eurofins WEJ Contaminants, Germany

Rapid dust screening by means of LC-MS/MS to obtain occurrence patterns of Fusarium, Aspergillus, Penicillium and Alternaria toxins in corn
Mareike Reichel, S. Staiger and S. Biselli
Eurofins WEJ Contaminants, Germany
P67 Co-occurrence of Fusarium, Aspergillus, Penicillium, Alternaria toxins and ergot alkaloids in European wheat and rye samples screened by means of dust analyses
Mareike Reichel, S. Staiger and S. Biselli
Eurofins WEJ Contaminants, Germany

P68 Detection of ochratoxin A contamination in stored wheat and barley using near-infrared (NIR) hyperspectral imaging system
Thiruppathi Senthilkumar1, D.S. Jayas1, N.D.G. White2, P.G. Fields2 and T. Gräfenhan3
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P69 Analysis of patulin in apple-based products using molecularly imprinted polymer sample preparation and fast UHPLC detection method
K. Espenschied, M. Ye and Olga I. Shimelis
MilliporeSigma, USA

P70 Effect of sample matrix on ionization effects during LC-MS analysis of mycotoxins in corn and animal feed
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P71 Analysis of mycotoxins in cereals using a simple extraction and LC-ESI/MS/MS with fast polarity switching and scheduled MRMs (multiple reaction monitoring)
Mike Roscoe, R. Blagden and S.A. Tittlemier
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P72 Optimized LC-MS method for multi-mycotoxin analysis in human plasma for exposure studies of Canadian population
Irina Slobodchikova, C.S. Monnin, S. Rahman, R. Sivakumar and D. Vuckovic
Department of Chemistry and Biochemistry, Concordia University, Canada

P73 Development of nanosensor platforms for mycotoxin detection
M.K.A. Kadir and Ibtisam E. Tothill
Advanced Diagnostics and Sensors Group, Cranfield University, UK

P74 Performance of grinders and dividers for preparing whole oats for mycotoxin analysis
J. Chan, W. Harnden, D. Bockru, Michael Tran, T. McMillan, D. Gaba and S.A. Tittlemier
Grain Research Laboratory, Canadian Grain Commission, Canada

P75 Application of biomolecular methods for the quantification of mildew damage and its causal agents in red spring wheat from Western Canada
Michael Tran, S.K. Patrick, J. Bamforth, M. Roscoe, S.A. Tittlemier and T. Gräfenhan
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P76 Development of an immunoaffinity column for sterigmatocystin analysis using an organic solvent-tolerant monoclonal antibody
Mikiko Uchigashima, K.Ogura, A. Bansho
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P77 Development and application of an accurate mass LC-MS/HRMS library for the screening of mycotoxins and other fungal metabolites in food and feed
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Xinwen Wang and S. Powers
VICAM A Waters Business, USA
P79 Single/multi-laboratory evaluations of mycotoxin analysis in foods by liquid chromatography-mass spectrometry
Kai Zhang, J.W. Wong, A.J. Kryntotsky and T.H. Begley
Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, USA

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P80 Biocontrol of Aspergillus flavus: the Italian experience
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P81 High moisture corn grain ensiling is a process conducive to fumonisins B1 biodegradation
C. Martinez Tuppia1, V. Atanasova-Penichon1, S. Chéreau1, M. Castex1, Pascal Drouin2, N. Ponts1, J.-M. Savoie1 and F. Richard-Forget1
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P82 Reduction of ergopeptide alkaloid concentrations in ergotized grasses by hay production
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P83 Modeling support for minimizing aflatoxin B1 contamination in dairy cattle compound feed
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P84 Inactivation of aflatoxicogenic Aspergillus flavus by photosensitisation with natural plant products
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David Ioi1,2, T. Zhou1, M.F. Marcone2 and R. Tsao1
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P86 Critical point sources of ochratoxin A contamination in on-farm stored winter wheat
Victor Limay-Rios and A. Schaafsma
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P87 Efficacy of a fungal and bacterial antagonist for controlling growth, FUM1 gene expression and fumonisin B1 by Fusarium verticillioides on corn cobs of different ripening stages
N.I.P. Samsudin, A. Rodriguez, A. Medina and Naresh Magan
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P88 Pre- and post-harvest management practices for minimization of mycotoxin contamination: a baseline study of knowledge, attitude and practices of subsistence farmers in Shamva and Makoni districts, Zimbabwe
Lucia R. Manema1, T.C. Murashiki2, D. Maringe1, T. Nyanhete3, C.P. Ambali4, C. Chidewe2 and L.K. Nyanga1
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C. Verheecke, P. Anson, D. Caron, T. Liboz and Florence Mathieu
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Critical control point-based mitigation of Fusarium mycotoxin production in stored grains of Job’s tears (Coix iachryma-jobi L.)
J. Kim¹, T.-J. An² and Yuseok Moon¹
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Effect of cropping measures on occurrence of Fusarium species and mycotoxins in grain corn – results from a multi-year survey in Switzerland
Tomke Musa¹, E. Jenny¹, U. Buchmann², J. Hiltbrunner², M. Sulyok³, H.-R. Forrer¹ and S. Vogelsgang¹
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Fate of enniatins and deoxynivalenol during pasta cooking
Monique de Nijs¹, H. van den Top¹, H. Mol¹, J. de Stoppelaar² and P. Lopez¹
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Comparing different methods of field application of bacterial endophytes as biocontrol agents to combat Gibberella ear rot in corn
Charles Shearer, V. Limay-Rios and M.N. Raizada
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Biological control of ear rot (Aspergillus flavus) on sweet corn using Trichoderma harzianum
Benice J. Sivparsad, N. Chiuraise and M.D. Laing
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Alaa Baazeem, A. Rodriguez, A. Medina and N. Magan
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The impact of climate change on free and masked fumonisins
Chiara Dall’Asta¹ and Paola Battilani²
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Fungi and mycotoxins in cheese – modeling the ecological needs
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rtfA, a putative RNA-pol II transcription elongation factor gene, is necessary for normal morphological and chemical development, proper response to oxidative stress and pathogenicity in Aspergillus flavus
Jessica M. Lohmar¹, P.Y. Harris-Coward², J.W. Cary², S. Dhingra¹ and A.M. Calvo¹
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Impact of climate change factors on growth and ochratoxin A production by Aspergillus section Circumdati and Nigri species on coffee
A. Akbar, A. Medina and Naresh Magan
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P100 Identification and classification of agronomic factors involved in ergot and its alkaloids content in small grain of cereals
Béatrice Orlando, C. Maumene, R. Valade, L. Maunas, N. Robin and L. Bonin
ARVALIS - Institut du végétal, France

P101 Fungal biotransformation of chlorogenic and caffic acids by Fusarium graminearum – new insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals
V. Atanasova-Penichon, M.-N. Verdal, G. Marchegay, L. Pinson-Gadais, C. Ducos and Florence Richard-Forget
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P102 The functional characterization of rmta, an arginine methyltransferase in the plant pathogen Aspergillus flavus
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P103 Relationship between ochratoxin A accumulation and pathogenicity of Aspergillus spp. in grapes
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P104 Patulin in ‘Galaxy’ apple – effect of different controlled atmosphere storage conditions and 1-MCP application
I.D. dos Santos1, I.R. Pizzuti1, Martien Spanjer2, M.E.Z. Fontana1, J.V. Dias1, L.N. Marques3, A. Brackmann4, R. de Oliveira Anese4 and F.R. Thewes4
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P105 An in vitro experiment to determine the levels of ochratoxin a produced under controlled temperature and moisture conditions in wheat kernels
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P106 Food system-wide preliminary survey of factors associated with mycotoxin risk in Aurepalle, Telangana, India
Anthony J. Wenndt and R.J. Nelson
Plant-Pathology and Plant-Microbe Biology, Cornell University, USA

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P107 Effects of fingerroot extracts on aflatoxin B1 epoxide formation and mutagenicity
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P108 Efficacy of UNIKE® Plus to reduce the toxicity of a combination of aflatoxin B1 and fumonisin B1 in weaning pigs
T.A. Shannon1, M.C. Shannon1, G. E. Rottinghaus1, T. J. Evans1, D. Y. Kim1, D. R. Ledoux1 and Radka Borutova2
1University of Missouri, USA and 2Nutriad International, Belgium
P109 Aflatoxin response metabolites from Vibrio gazogenes – a novel system for generation of aflatoxin synthesis inhibitors

Anindya Chanda¹, P.M. Gummadidala¹, Y.-P. Chen¹, K. Beauchesne², K. Miller¹, C. Mitra¹, N. Banaszek¹, M. Velez-Martinez¹, P.D.R. Moeller², J.L. Ferry³ and A.W. Decho¹
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P110 Biodegradability of mycotoxins in anaerobic digestion: a new solution to valorize contaminated mycotoxin batches?

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P111 On the pursuit of mycotoxins degrading enzymes – a straightforward framework based on a joint in silico/in vitro approach

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P112 Anti-mycotoxin additives evaluated in animals

Paulo Dilkin¹, D. Liberalesso¹, R.D. Vargas¹, A.O. Mallmann¹, L.R.S. Wovst², C.A.A. Almeida¹, L.Z. Giacomoini², D.L. Frantz³, N.H.R. Anoni² and C.A. Mallmann¹
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P113 Partial effectiveness of a detoxifying agent to prevent deleterious effects of dietary zearalenone on sexually immature gilts

Eduardo M. Gloria, M. Sbardella, V.S. Miyada, M.A. Calori-Domingues, N. Yumi and B.J. De Sá
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P114 Application of fumonisin carboxylesterase FumD as a feed additive (FUMzyme®) counteracts the toxic effect of fumonisin B1 contaminated feed in pigs

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P115 Deoxynivalenol epimerization: an effective route for detoxification with potential agricultural and industrial applications

Yousef I. Hassan, X.-Z. Li, H. Zhu and T. Zhou
Guelp hospitality Food Research Centre, Agriculture and Agri-Food Canada, Canada

P116 Mycotoxicosis in sows, piglets and pigs

Astrid Koppenol¹, M.D. Sims² and G.C. Alberton³
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P117 Evaluation of circulating lymphocytes and gut health in T-2 mycotoxin contaminated broilers

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P118 Determination of blood and immune parameters in broilers exposed to fumonisin

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P119 Effects of the supplementation of yeast cell wall (Safmannan) on the blood serum level of zearalenone, α-zearanol, and β-zearanol in dairy cows fed a zearalenone-contaminated diet

J.C. Del Rio, M.C. Espejel, R. Marquez, A. Garcia and Virginie Marquis
Phileo Lesaffre Animal Care, France
**P120** Effects of yeast cell wall on zearalenone toxicosis in prepubertal female gilts
D. Shi1, W. Li2, B. Zhang2, Virginie Marquis3 and C. Ji1
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**P121** Aflatoxin contamination and the use of additives alter plasma parameters in ducklings – multiple trial analyses
Clémentine Oguey1 and G. Benzoni2
1Pancosma SA, Switzerland and 2InVivo NSA, France

**P122** Degradation of zearalenone using Saccharomyces cerevisiae NCYC R404
N. Schroeder, Jog Raj, L. Norton, S. Mann and R. Breitsma
Micron Bio-Systems Ltd., UK

**P123** Enzymatic detoxification of mycotoxins within the bioethanol production process
D. Kotz1, S. Rose1, Christina Schwab2, D. Schatzmayr1, S. Trupia3 and G. Schatzmayr1
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**P124** Efficacy of an algoclay-based adsorbent on breeding ducks exposed to polycontamination of mycotoxins
Si Trung Tran1, M. Gallisso1 and V.K. La2
1Olmix SA, France and 2Institute of Animal Sciences of South Vietnam, Vietnam

**P125** Microbial degradation of deoxynivalenol
Ilse Vanhoutte1, K. Audenaert1,2, S. De Saeger3 and L. De Gelder1
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**P126** Use of Actinomycetes for aflatoxins biodegradation
Carol Verheecke, P. Anson, Y. Zhu, T. Liboz and F. Mathieu
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**P127** Using enzymes and microorganisms to modify the mycotoxin deoxynivalenol
Nina Wilson1, D. Gantulga1, N. McMaster1, S. McCormick2, R. Senger3 and D. Schmale1
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**MISCELLANEOUS TOPICS**

**P128** Metabolomics – what does grain chemistry tell us about aflatoxigenic Aspergillus flavus?
Titilayo D.O. Falade1, P. Chrysanthopoulou2, M. Hodson2, R. Darnell2, J. Harvey1,4, M. Fletcher1, Y. Sultanbawa1, S. Were3, S. Korie6 and G. Fox1
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**P129** Risk ranking of chemical hazards in spices and herbs
E. van Asselt, J. Banach and Ine van der Fels-Klerx
RIKILT Wageningen UR, the Netherlands

**P130** Xenobiotic remediation in niche domination of soil-borne mycotoxigenic fungi – the deletion analysis of fungal lactamases in Fusarium verticillioides
Scott E. Gold1, M. Gao1,2, N.J. Crenshaw3 and A.E. Glenn1
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P131 Microorganisms associated with poor baking quality of wheat
Ingerd Hofgaard1, S. Koga2, H.U. Aamot1, U. Böcker2, E. Lysøe1, G. Brodal1, R. Dill-Macky1,3 and A.K. Uhlen2,4
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P132 MyToolBox – Safe food and feed through an integrated ToolBox for mycotoxin management
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OCCURRENCE
P1 – P21

P1: The epidemiology of *Fusarium langsethiae* in oats

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The plant pathogenic fungus *Fusarium langsethiae* produces the highly potent mycotoxins HT-2 and T-2. Since these toxins are frequently detected at high levels in oat grain lots, they pose a considerable risk for food and feed safety in Norway, as well as in other north European countries. To reduce the risk of HT-2/T-2-contaminated grain lots to enter the food and feed chain, it is important to identify factors that influence *F. langsethiae* infection and mycotoxin development in oats. However, the epidemiology of *F. langsethiae* is unclear. A three-year survey was performed to reveal more of the life cycle of *F. langsethiae* and its interactions with oats, other *Fusarium* species, as well as insects, mites and weeds. We searched for inoculum sources by quantifying the amount of *F. langsethiae* DNA in weeds, crop residues, and soil, sampled from a predetermined selection of oat-fields. To be able to define the onset of infection, we analyzed the amount of *F. langsethiae* DNA in oat plant material sampled at selected growth stages (between booting and maturation), as well as the amount of *F. langsethiae* DNA and HT-2 and T-2 toxins in the mature grain. We also studied the presence of possible insect- and mite vectors sampled at the selected growth stages using Berlese funnel traps. All the different types of materials were also analyzed for the presence *F. graminearum* DNA, the most important deoxynivalenol producer observed in Norwegian cereals, and which presence has shown a striking lack of correlation with the presence *F. langsethiae* in oat. Preliminary results show that *F. langsethiae* DNA may occur in the oat plant before heading and flowering. Some *F. langsethiae* DNA was observed in crop residues and weeds, though at relatively low levels. More results from this work will be presented at the meeting.

P2: Characterization of aflatoxigenic *Aspergillus* spp. and aflatoxin contamination of imported foods originated in Asia countries in Scotland

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The objective of this study was to evaluate the occurrence of aflatoxigenic molds and aflatoxins (AFs) in foods. Twelve commercial, dried Asian food commodities including jasmine brown rice, long grain rice, fragrant rice, peanut, green mung bean, black bean, black pepper, crushed chilli, five-spices mixed powder, white prune, salted plum pieces and white plum were collected in a local supermarket in Glasgow, UK and examined for mycological profile and contamination of aflatoxins. Eight samples (Jasmine brown rice, long grain rice, fragrant rice, peanut, black bean, black pepper, crushed chilli and five spice powder) were found to contain multiple genera of molds and 82 isolates were recovered. Of these, 55% were *Aspergillus* spp. and the rest were *Penicillium* spp. (15%), *Rhizopus* spp. (11%), *Mucor* spp. (3%), *Monascus* spp. (1%), *Eurotium* spp. (1%) and unidentifiable (18%). No molds or toxins were found in the remaining products. The highest level of mold contamination was observed in rice samples of which long grain rice revealed the highest number of mold isolates. *Aspergillus* spp. were detected in long grain rice, fragrant rice, peanuts, black beans and black pepper which were identified as *A. parasiticus* (aflatoxigenic), *A. versicolor*, *A. ustus*, *A. niger* and *A. ochraceus*. Analysis of aflatoxins by HPLC in food samples revealed that long grain rice, fragrant rice, peanuts, black beans and black pepper, which showed contamination of *Aspergillus* spp., contained undetectable aflatoxins. Jasmine brown rice and crushed chilli contained 14.7 and 11.4 µg/kg of AFs, respectively, in the absence of *Aspergillus*. AFB1 was detected in crushed chilli (10.7 µg/kg). In conjunction with the mycological profiles, it was found that the occurrence of aflatoxigenic molds did not correspond with the presence of aflatoxins in food samples. Jasmine brown rice and crushed chilli showed absence of *Aspergillus* spp. but they contained aflatoxins indicating that the mold was present at some stage during processing. The presence of aflatoxigenic *Aspergillus* in food commodities indicated the possibility that aflatoxins could be produced if food storage is poorly managed. These results emphasize the need for stricter control measures in reducing occurrence of *Aspergillus* and aflatoxins in foods.
P3: Prevalence, mycotoxin production ability and characterization of *Fusarium* species on Korean adlay (Coix lacrymal-jobi L.)

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The present study was carried out to examine the post-harvest fungal occurrence in adlay seeds, to isolate and identify most common *Fusarium* species by molecular and morphological methods and to investigate the toxin producing ability of isolated 10 *Fusarium* species. Adlay seed samples were collected from 3 regions of Korea in 2012. Fungi were isolated and identified from the seed samples and the dominated 7 fungal genera represented as *Alternaria*, *Cladosporium*, *Cochliobolus*, *Curvularia*, *Fusarium*, *Leptosphaerulina* and *Phoma*. Among them, 45.6% fungi were *Fusarium* species. Based on combined sequences of two protein coding genes, EF-1α, β-tubulin and phylogenetic analysis by the maximum likelihood (ML) method, 10 species of *Fusarium* were characterized and identified as *F. incarnatum* (11.67%), *F. kyushense* (10.33%), *F. fujikuroi* (8.67%), *F. concentricum* (6.00%), *F. asiaticum* (5.67%), *F. graminearum* (1.67%), *F. miscanthi* (0.67%), *F. polyphialidicum* (0.33%), *F. armeniacum* (0.33%) and *F. thapsinum* (0.33%). Collected *Fusarium* samples were investigated for toxin producing ability by ELISA quantitative analysis. The results confirmed that fumonisin was produced by *F. fujikuroi*, zearalenone by *F. asiaticum* and *F. graminearum*, and deoxynivalenole by *F. asiaticum*, *F. armeniacum*, *F. graminearum*, *F. incarnatum* and *F. kyushense*.

P4: Occurrence of *Fusarium* mycotoxins in Croatian cereals harvested in 2015

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This is the first report of non-regulated *Fusarium* mycotoxins occurrence in Croatian cereals. Several different *Fusarium* mycotoxins were detected in the cereal samples harvested during 2015 which were collected from all Croatian regions. Two hundred different cereal samples were collected: 97 corn, 64 wheat, 22 barley, 11 oat, 4 triticale and 2 rye samples. Analysis was performed using a liquid chromatography-tandem mass spectrometry multi-mycotoxin method. Of the legally regulated mycotoxins deoxynivalenol (DON), and zearalenone were most frequently detected. The maximum concentration of DON was 2,680 μg/kg in corn with 941 μg/kg of masked deoxynivalenol-3-glucoside. Other detected trichotheccenes were 3- and 15-acetyldeoxynivalenol, nivalenol, nivalenol-3-glucoside, T-2 toxin, HT-2 toxin, HT-2 glucoside, T-2 tetraol, diacetoxyscirpenol, 4-deacetylneosolaniol. In addition to zearalenone with a maximum concentration of up to 618 μg/kg in corn, zearalenon-4-sulfate, and α- and β-zearalenol were also detected. Several fumonisins were also present: fumonosin B1, B2, B3, B4, and hydrolyzed fumonosin B1. Other detected *Fusarium* metabolites included beauvericin, enniatins (A, A1, B, B1, B2, and B3), moniliformin, equisetin, epi-equisetin, metilequisetin, aurofusarin, rubrofusarin, apicidin, culmorin, 5- and 15-hydroxyculmorin, 15-hydroxyculmorin, butenolide, chrysogine, fusaric acid, fusapyron, deoxyl fusapyron, fusaproliferin, and fusarin C. Acknowledgments. This research was financially supported by the European Structural and Investment Funds, European Social Fund (ESF), Power of Development – Human resources development, CroMycoscreen HR.3.2.01-0274.

P5: Occurrence of aflatoxins in farming fish from São Paulo State, Brazil

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Fish feed is susceptible to contamination by fungi and consequently can contain mycotoxins. Aflatoxins (AF) can be deleterious to fish production, affecting the development of fish, besides can accumulate in fish muscle and be ingested by humans. The aim of this study was to detect and quantify aflatoxins in feed and fish of farming fish from São Paulo State, Brazil. Stored feed and feed in use were obtained from five farming fish, adding up to 11 samples. Aflatoxin B1, B2, G1 and G2 were analyzed by high performance liquid chromatography (HPLC). Two species of fish were sampled: Pacu (*Piaractus*...
Brycon cephalus

Scientific literature comparing the content of deoxynivalenol (DON), whether organically crops are more prone to mycotoxin contamination than conventionally grown crop has been questioned whether the farming system has an impact on development of mycotoxins, and temperature and humidity), host species.

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Fungal infection and production of mycotoxins in plants depend on weather conditions (mainly temperature and humidity), host species and cultivars, agronomy and other environmental conditions. It has been questioned whether the farming system has an impact on development of mycotoxins, and whether organically crops are more prone to mycotoxin contamination than conventionally grown crops. Scientific literature comparing the content of deoxynivalenol (DON), HT2+T2 toxins, zearalenone (ZEN),

mesopotamicus) and Matrinxã (Brycon cephalus). In each farming fish, one kg of each species was collected to constitute a representative pool sample. AFB1 and AFM1 were quantified in muscle and liver by HPLC. Results revealed that all feed samples in use were found to contain AFB1. AFB1 was detected in 90.9% of the samples, presenting a mean level of 0.22 µg/kg and a maximum level of 1.42 µg/kg, while AFB2 was found in 81.8% of the feed samples, with a mean level of 0.06 µg/kg. AFG1 was detected in 18.2% of the feed samples, with a mean of 0.12 µg/kg, and AFG2 in 45.5% of samples, with a mean level of 0.24 µg/kg and a maximum of 1.18 µg/kg. AFB1, AFB2, AFG1 and AFG2 were found together in 18.2% of the samples, presenting a mean of 1.42 µg/kg. AFB1 was detected in 60% of the Pacu muscle samples, in levels below 0.01 µg/kg. AFM1 was detected in 80% of the Pacu muscle samples, in levels from 0.12 to 0.81 µg/kg. Considering Matrinxã fish, AFB1 and AFM1 were found in 100% of the muscle samples, in levels ranging from 0.03 to 0.61 µg/kg and from 0.02 to 5.87 µg/kg, respectively. All liver samples presented AFB1 and AFM1 for both species. Pacu liver presented AFB1 levels from 0.04 to 0.19 µg/kg and AFM1 from 0.28 to 6.67 µg/kg, while Matrinxã liver ranged from 0.21 to 12.67 µg/kg for AFB1 and from 0.68 to 11.80 µg/kg for AFM1. It is concluded that, despite low aflatoxins levels found in fish feed, it was possible to detect AFB1 and AFM1 in fish muscle of the two species analyzed. Therefore, it is necessary to control aflatoxins levels in fish feed to avoid transfer to fish muscle.

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P6: Frequency and concentration of aflatoxins and fumonisins in corn in Kenya and Tanzania: implications for food safety

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Stored corn from farms in Kenya (n=478) and Tanzania (n=100) was sampled during 2012/13 to estimate the frequency and concentration of aflatoxins and fumonisins in relation to the legally acceptable limits. The toxins were analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Aflatoxin B1 was detected in 30.2% of samples (limit of detection (LOD) 0.2 µg/kg) and fumonisin B1 was detected in 46.6% of samples (LOD 1.2 µg/kg), with concentrations up to 6,075 µg/kg aflatoxin B1 and 6,103 µg/kg fumonisin B1 recorded. Aflatoxins and fumonisins co-occurred in 17.5% samples. Both the occurrence rates and concentrations of the toxins varied significantly between and within the two countries surveyed. In reference to the standards for acceptable limits for aflatoxin B1 (5 µg/kg), total aflatoxins (10 µg/kg) and fumonisin (2 µg/kg) for the two countries, 18.7% of the corn was unfit for human consumption, with 16.2% exceeding the aflatoxins limit and 3.6% fumonisin limit. Only 0.9% exceeded both limits. The results provide a sobering picture of the mycotoxin problem, indicating the magnitude of in-field contamination. Due to their persistent nature, the contamination levels of these mycotoxins tends to increase without proper postharvest storage practices and technologies. Corn has a central role in the culture and diet in East Africa; the health implications of mycotoxin contamination are therefore of great concern to health and agriculture authorities. Clearly, strategies to reduce the levels of both pre- and post-harvest contamination of corn in the East African corn food chain are needed urgently. Further studies exploring factors that influence in-field mycotoxin contamination are on-going.

P7: Assessment of mycotoxins in organically and conventionally grown crops in temperate regions

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Fungal infection and production of mycotoxins in plants depend on weather conditions (mainly temperature and humidity), host species and cultivars, agronomy and other environmental conditions. It has been questioned whether the farming system has an impact on development of mycotoxins, and whether organically crops are more prone to mycotoxin contamination than conventionally grown crops. Scientific literature comparing the content of deoxynivalenol (DON), HT2+T2 toxins, zearalenone (ZEN),
nivalenol (NIV), ochratoxin A (OTA) and fumonisin (FUM) in cereal grains, and patulin (PAT) in apple and apple-based products, produced from organically and conventionally grown crops in temperate regions have been reviewed. The review is based on a risk assessment from the Norwegian Scientific Committee on Food Safety (2014) and supplemented with some recently published data. Some of the studies are based on data from controlled field trials, however most are farm surveys, and some are food basket surveys. In total, 46 comparisons of DON in cereal grains (29 in wheat) were included. The majority of the studies found no statistically significant differences in DON content in grain from the two farming systems, but several studies showed a tendency of lower DON content in organically than in conventionally produced cereals. A number of the investigations reported low DON levels in grain, far below the EU limits for food. Many authors suggested that weather conditions, years, locations, crop rotation and tillage practice were more important for the development of DON than the type of farming. Organically produced oats mainly contained lower levels of HT2+T2 toxins than conventionally produced oats. Most studies on ZEN reported no differences between farming systems, or lower concentrations in organically produced grain. For the other mycotoxins in cereals, mainly low levels and no differences between the two farming systems were reported. Some studies reported on higher PAT contamination in organically than in conventionally produced apple and apple products. The difference may be due to more efficient disease control in conventional orchards. It cannot be concluded that any of the two farming systems increases the risk of mycotoxin contamination. Despite no use of fungicides, an organic system appears generally able to maintain mycotoxin contamination at low levels. More systematic comparisons from scientifically controlled field trials and surveys are needed to clarify if there are differences in the risk of mycotoxin contamination between organically and conventionally produced crops.

P8: The occurrence of mycotoxins in Polish silage – a preliminary study

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Silage is one of the important winter feeds for ruminants, produced from ensiled crops like grasses, corn and legumes. Unfortunately, silage can become contaminated with various mycotoxins which can contaminate the silage before ensiling (pre-harvest) or after ensiling (post-harvest). The toxicity of the mycotoxin for the animals required a survey of its levels it this kind of feed. The aim of the study was a determination of mycotoxins in silage samples by LC-MS/MS method. The samples (n=122) were collected by Veterinary Inspection across the Poland in 2015. The sample was stored (<-18°C) and homogenized before analysis. The analytes (beauvericin, diacetoxyscirpenol, nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, HT-2 toxin, T-2 toxin, ochratoxin A, fumonisin B1 and B2, aflatoxin B1, fusarenon-X, enniatins: A, A1, B, B1; sterigmatocystin and zearalenone) were extracted from the silage sample (5 g) with mixture (20 ml) of acetonitrile:water: formic acid (79:20:1) in 30 min. Next, the sample was centrifuged and cleaned up with a C18 sorbent (50 mg) and magnesium sulphate (150 mg). Extracts were evaporated, mixed with labelled internal standards solution (used for quantitation) and determined with UHPLC-MS/MS technique. Mycotoxins were separated with Kinetex Biphenyl column (100x2.1mm, 2.7 µm, Phenomenex) with 15 min, gradient elution of mobile phase consisted of methanol, 0.01 M ammonium acetate (pH 6.8). The mass spectrometer was operated in electrospray positive (ESI+) and negative (ESI-) ionization mode and two multiple reaction monitoring (MRM) transitions for each analyte were monitored. The method performance allows determining mycotoxin above limit of detection in the range 1-10 µg/kg depending on the analyte. The occurrence of regulated mycotoxin in samples was the following: deoxynivalenol, 68% (in the range 27-4.347 µg/kg ), nivalenol, 46% (30-14.262 µg/kg), fumonisn B1, 56% (4.0-379 µg/kg), zearalenone, 43% (3.0-444 µg/kg), and HT-2, 37% (9.0-107 µg/kg). The occurrence of enniatins (A, A1, B, B1) was in the range 66-89% and beauvericin 87%. Overall, over 60% of the samples contained more than 5 determined mycotoxins. This preliminary result shows significant contamination of silage in terms of concentration and number of mycotoxins. There is a need for future correlation of these results with other factors describing silage production like pH, time and way of storage, presence of silage additives, etc.
P9: Detection of aflatoxin B1 contamination in imported chia seed dietary supplements in Thailand

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Chia seed (Salvia hispanica) dietary supplements have been a healthy trend going around for a while now in Thailand due to their possible health promoting effects. As plant oil seeds they can be contaminated with mycotoxins, especially aflatoxin B1 (AFB1) at various stages. The aim of this study was to determine the possibility of contamination of AFB1 in imported chia seed dietary supplements consumed in Thailand. The QuEChERS procedure and high performance liquid chromatography with fluorescence detection (HPLC-FLD) using pre-column derivatization were used for analysis of AFB1. The average recoveries for AFB1 ranged from 75.4-108.2% at spiked levels of 2-25 ng/g with relative standard deviations (RSDs) lower than 11%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.6 and 1.2 ng/g, respectively. A preliminary survey was performed on 30 samples consisting of 10 brands of imported chia seed dietary supplements that were collected from supermarkets and health food stores in Bangkok during September 2015 to January 2016. Three chia seed samples were contaminated with AFB1. The mean AFB1 levels in the three samples were 1.3, 2.8, and 3.2 ng/g, respectively. Although all samples had mean concentration of AFB1 below the maximum permissible limits in Thailand (all foods at 20 ng/g), two chia seed samples were contaminated with levels higher than the European Union regulatory limits for AFB1 (cereal products, 2 ng/g). The results showed that chia seed dietary supplements sold in Thailand require monitoring for AFB1 contamination. The consumption of dietary supplements contaminated with harmful AFB1 may be a threat to the health of populations.

P10: A global survey of regulated, masked and emerging mycotoxins and secondary metabolites in agricultural products from 2012-2015

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Increasing global trade of agricultural commodities, an increasing number of extreme weather events and a changing climate necessitate global monitoring of mycotoxin and secondary metabolite concentrations. While regulated mycotoxins are monitored on a regular basis, data for emerging and masked compounds are still scarce. For this survey, 716 samples of finished feed from 44 countries, 276 corn samples (34 countries) and 139 corn silage samples (17 countries) collected between 2012 and 2015 were analyzed. Specifically, concentrations of 59 mycotoxins and secondary metabolites were investigated (e.g., A and B trichothecenes, enniatins and beauvericin). Concentration data were acquired using a single LC-MS/MS multi-mycotoxin method to ensure comparability. Results from this global data set suggest that deoxynivalenol (DON) and zearalenone (ZEN) continue to be of concern with a relatively large number of highly contaminated samples. Fumonisins (FUM) were found at important concentrations in samples from Southern Europe, South America and South Africa. Large increases in yearly average DON concentrations, especially in Europe were observed in 2014 and 2015. Aflatoxins were of concern in African and Eastern European samples with concentrations as high as 60 and 90 μg/kg, respectively. Some emerging and masked compounds showed high concentrations for individual samples, e.g., in the 400 to 1,400 μg/kg range for enniatins. High levels of aflatoxin precursors, e.g., 500 to 1,700 μg/kg were seen in some samples. Overall aflatoxin precursors were detected in less than 20% of all samples, including corn and corn silage. South African corn showed significant concentrations of moniliformin and beauvericin. Good correlation was observed for co-occurring DON and DON-3-glucoside and ZEN and ZEN-4-sulfate. Aflatoxins and its precursors were strongly correlated in samples from Africa and South America. Correlation of T-2 toxin and DON with beauvericin in finished feed samples was also observed in Eastern European samples. Monitoring and comparable acquisition of regulated, masked and emerging compound concentrations in agricultural samples needs to be expanded to further identify trends in concentrations and co-occurrence.
Feed raw materials show different profiles of mycotoxin contamination depending on the type of culture and storage conditions. Corn is known as a major source of mycotoxins and particularly of fusariotoxins as described in 2003 by SCOOP survey. The aim of the present study is to identify the occurrence of fusariotoxins in different types of corn materials: dry corn grain, humid corn grain (silage corn grain) and corn silage (fermented full corn plant). This study uses the LABOCEA database composed of chromatography analyses run with LC-MS/MS from 2013 to 2015. 24 fusariotoxins are tested in each analysis. The percentage of positive samples (>LOQ) and the median level of contamination (µg/kg) per mycotoxin are the two main criteria used. In order to avoid any geographical interaction, samples from one restricted area only (France) are considered: dry corn grain (n=337), humid corn grain (n=119) and corn silage (n=557). Data shows that all types of corn materials are polycontaminated with fusariotoxins (per sample, in average 7 fusariotoxins are positive) and that deoxynivalenol (DON) is the most frequent (>90% of positive) and the most present (median value >600 µg/kg) fusariotoxin. The profiles of fusariotoxins (% of positive samples) are very similar between corn raw materials whereas the level of median contamination depends on the type of corn material. Corn silages have higher DON median level of contamination (1,090 µg/kg) than humid corn grains (980 µg/kg) than dry corn grains (720 µg/kg). Deoxynivalenol acetyl forms (15-O-acetyl and 3-O-acetyl DON) contaminations are similar for all types of corn materials. Nevertheless, nivalenol (NIV) median level in corn silage is 4 times higher than in corn grain (290 vs. 68 µg/kg, respectively). On the contrary, dry corn grains have higher median sum of fumonisins (320 µg/kg) than corn silage (40 µg/kg) and humid corn (68 µg/kg). Zearalenone (ZEN) occurrence is similar in all types of corn materials with contamination levels far lower than for DON (median level: <200 µg/kg for ZEN; >700 µg/kg for DON). Regarding type A trichothecenes, the profiles of contamination are equivalent for all types of corn materials apart for one metabolite (MAS) that is more often present (% positive samples) in corn silage (66.4%) than in humid corn grain (32.8%) and in dry corn grain (11%). Some parameter, such as time of harvest and type of preservation, may explain the variable profile of fusariotoxins among corn materials.

**P12: Comparative analysis of fusariotoxins occurrence in wheat, barley and corn grain**

Feed raw materials show different fusariotoxins occurrence depending on the type of culture, as shown by SCOOP survey in 2003. The aim of this study is to identify the occurrence of fusariotoxins in the 3 main cereals used by the feed industry: wheat, barley and corn grain. This study uses the LABOCEA database composed of chromatography analyses run with LC-MS/MS from 2013 to 2015. 24 fusariotoxins are tested in each analysis. The percentage of positive samples (>LOQ) and the median level of contamination (µg/kg) per mycotoxin are the two main criteria used. In order to avoid any geographical interaction, samples from one restricted area only (France) are considered: wheat (n=274), barley (n=104) and corn grain (n=336). Data shows that corn grain is more polycontaminated than wheat and barley (per sample, in average 7 fusariotoxins are positive for corn grain, 2 for wheat and 3 for barley). As a consequence, the percentage of positive samples per mycotoxin is more important in corn grain than for other cereals. Deoxynivalenol (DON) is the most frequent fusariotoxin (>90% of positive) in all cereals, but its median level of contamination is far higher for corn grain (740 µg/kg) than for wheat (215 µg/kg) and for barley (75 µg/kg). The levels of 15-O-acetyl DON, zearalenone (ZEN) and fumonisins are also significantly higher for corn grain (153, 135 and 345 µg/kg, respectively) than for wheat (15, 25 and 50 µg/kg, respectively) and barley (20, 25 and 30 µg/kg, respectively). Focusing only on straw cereals, wheat shows higher median contamination in DON, T-2 toxin and fumonisins (215, 35 and 50 µg/kg, respectively) than barley (75, 10 and 30 µg/kg, respectively), whereas barley is more often contaminated (% of positive samples) in DON acetyl forms (40.4% in 15-O-acetyl and 17.3% in 3-O-acetyl DON) than wheat (5.8% in 15-O-acetyl and 16.4% in 3-O-acetyl DON). The different cropping parameters (time of harvest, use of fungicide, etc.) of corn could explain the important differences in fusariotoxins occurrence compared to straw cereals. *Fusarium* strains have different developments between wheat and barley and thus may explain the variable fusariotoxins occurrence.
P13: A UK total diet study of mycotoxins

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A total diet study (TDS) is representative of the whole diet. A TDS is different from many surveys in that foods are prepared for consumption (rather than being analyzed as sold) before being pooled into groups before analysis. The UK Food Standards Agency (FSA) funded this TDS on mycotoxins. Samples for 138 categories of foods defined by the FSA were purchased from 24 local authorities (a total of 3312 samples). The categories were classified under twenty eight food groups and seventeen of these were analysed in the mycotoxin study. These included bread, miscellaneous cereals, offal, oils and fats, eggs, sugars and preserves, potatoes, other vegetables, fresh fruit, fruit products, non-alcoholic beverages, milk, dairy products, nuts, alcoholic drinks, snacks and sandwiches. The samples were analyzed for a range of mycotoxins; aflatoxins, ochratoxin A, fumonisins, patulin, zearalenone, trichotheecenes, sterigmatocystin, ergot alkaloids, citrinin, cyclopiazonic acid and moniliformin using methods designed to give low limits of quantification. In addition prototype immunoaffinity columns for citrinin were used in conjunction with LC-MS/MS for the first time in this study. Seventeen food group samples were prepared by combining the individual category samples from within that group. These were analyzed for mycotoxins to compare with mathematical calculations carried out on the results for the food categories to check the homogeneity of the food groups. The most frequently detected toxins were deoxynivalenol and ergot alkaloids which were detected in all bread samples and sandwiches as well as other cereal products. None of the samples exceeded any maximum permitted limit. Very few residues of any of the other mycotoxins analyzed were found in the samples tested, most results were below the limits of quantification which were as low as technically achievable, and typically in the sub or low µg/kg range. This is the first UK TDS study for mycotoxins. These results show very little incidence of mycotoxins in UK food samples, with very few results above the low limits of quantification. The data can be used for future intake calculations to calculate background exposure to various mycotoxins from the whole diet and also to compare exposures to those calculated by other sources.

P14: Comparative metabolism study of HT-2 and T-2 toxin in small grain cereals

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The type A trichothecene mycotoxins HT-2 toxin (HT-2) and T-2 toxin (T-2) are mainly produced by Fusarium sporotrichioides, F. langsethiae and F. poae. Small grain cereals, especially oats, but also barley and wheat are frequently contaminated with HT-2 and T-2 (Lattanzio et al., 2012). However, only limited knowledge exists about the natural occurrence of HT-2 and T-2 derived plant metabolism products. Since the native mycotoxins as well as its metabolites in plants may contribute to toxicological effects, affected food and feed may constitute a health risk for humans and animals. The objective of this study was to obtain a comprehensive picture of the metabolism of HT-2 and T-2 in oats, barley and wheat. Experimental design for all investigated plant species included untargeted screening based on stable isotope labelling, liquid chromatography-high resolution mass spectrometry (LC-HRMS) measurements and data processing by MetExtract II software to detect all HT-2 or T-2 derived metabolites. As a result, novel glucosylated forms of the toxins, malonylglucosides, as well as acetyl and feruloyl conjugates were annotated. Secondly, a quantitative time course experiment was performed to determine metabolite formation and estimate mass balances as a function of time. These data showed that the metabolic fate of both toxins overlaps to a great extent, since T-2 is rapidly transformed into HT-2 in planta. It is assumed that glucosylation is an important plant detoxification mechanism, since HT-2 glucoside was a main metabolite in all investigated plants species which reached its maximum already 1 day after toxin treatment. In comparison, the highest conversions of the native toxins into HT-2 glucoside were observed in oats. The decrease of its concentration at later time points indicates that HT-2 glucoside is the initial substance for many further metabolism steps, as additional conjugations or group losses. In T-2 treated plants, a second, fast metabolism route was observed, namely the conjugation of acetic or tentative ferulic acid to the intact T-2 backbone (Meng-Reiterer and Varga, 2015; Nathanail et al., 2015). This might be an interesting finding, since there are some indications in the literature for the involvement of phenolic acids, as ferulic acid, in resistance
Sterigmatocystin is a mycotoxin produced by fungi of the genus *Aspergillus* that can occur in cereals and cereal-based products as well as certain other food products. *A. versicolor* is the most common source in food. Sterigmatocystin shares its biosynthetic pathway with aflatoxins. *A. versicolor* seems unable to metabolize sterigmatocystin into O-methylsterigmatocystin, the direct precursor of aflatoxin B1 and G1. As a consequence, food commodities infested by this fungus can contain high amounts of sterigmatocystin, whereas infection by *A. flavus* and *A. parasiticus* results in low amounts of sterigmatocystin because most is converted into aflatoxins. Sterigmatocystin has an aflatoxin-like structure and has been categorized by the International Agency for Research on Cancer (IARC) as a class 2B, possible human carcinogen. A lack of suitable data on the occurrence of sterigmatocystin did not allow proper characterization of its risk for human health. This was the trigger for the European Food Safety Authority (EFSA) to initiate an extensive survey on the occurrence of sterigmatocystin in the EU.

In total, 1,259 samples were collected at processing plants, storage facilities, wholesale and retail. This was done between August 2013 and November 2014, in nine European countries. The products originated from 27 European countries and 18 other countries. All samples were analyzed by liquid chromatography with tandem mass spectrometry. The LOQ was 0.5 µg/kg and the LODs were in the range 0.05-0.15 µg/kg (0.005-0.01 µg/l for beer). Overall, sterigmatocystin was identified in 10% of the samples. Sterigmatocystin was not detected in beer and nuts. More than 50% of the contaminated samples contained levels between LOD and LOQ. In other cases, levels were between 0.5-6 µg/kg with one exception (33 µg/kg in oats). Rice and oats were identified as the cereals most prone to sterigmatocystin contamination. In cereal products, levels were lower than in cereal grains. The highest incidence was in breakfast cereals (19%). In the contaminated cereal products, rice and oats were often present as ingredients. Acknowledgments. This work was supported by the European Food Safety Authority (EFSA) through grant GP/CONTAM/2013/02-GA1, and co-financed by the Dutch Ministry of Economic Affairs (WOT-02-001-061) and the Food Standards Agency (UK). The full report is available as EFSA supporting publication 2015:EN-774, http://www.efsa.europa.eu/it/supporting/pub/774e. The sole responsibility of the content lies with the authors. EFSA is not responsible for any use that may be made of the information contained therein.

**P16: Worldwide mycotoxin occurrence in feeds and raw materials – survey data 2015**

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Mycotoxins are toxic secondary metabolites produced by certain fungi such as *Penicillium*, *Fusarium* and *Aspergillus*. Mycotoxin-producing fungi damage crops, causing severe economic losses to food and feed production. Moreover, many mycotoxins impair immunity and organ functions, causing loss of productivity, diseases and death in humans and animals that consume contaminated food or feed. Mycotoxin occurrence in all kinds of commodities is a worldwide phenomenon. BIOMIN conducts an annual mycotoxin survey. The focus of this study is to evaluate the extent of mycotoxin contamination in various feed samples. In the year 2015, more than 8,000 samples sourced from around the world were analyzed for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin (T-2), fumonisins (FUM) and ochratoxin A (OTA). The majority of samples originated from Europe (3,862), Asia (2,484) and from the Americas (1,586). Samples were analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). In total, 84% of all samples contained at least one of the five main mycotoxins. Afla was present in 18% of all samples at mean concentration of 40 µg/kg. ZEN was present in 56% of samples at mean concentration of 253 µg/kg. DON was detected in 73% of all samples at mean concentration of 1,090 µg/kg. T-2 was found in 23% at mean concentration of 26 µg/kg. FUM in 61% of all samples at mean concentration of 1,089 µg/kg. OTA in 18% of samples at mean concentration of 7 µg/kg. DON was the most common mycotoxin in 2015 and was found in the majority of samples. The very high average concentration of DON represents a significant risk to livestock. Mycotoxin co-occurrences were identified in 53% of samples, this presence of two or more...
Mycotoxins can result in synergistic or additive toxic effects on animals consuming the affected material. The survey results indicate that mycotoxins remain a serious concern in agricultural production. *Fusarium* produced mycotoxins are the most common. An effective mycotoxin risk management program should be applied to protect animals from negative effects of mycotoxins.

**P17: The hidden threat – emerging mycotoxins**

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Simultaneous detection and quantification of a broad spectrum of mycotoxins has facilitated the screening of a larger number of samples and thereby placing emerging mycotoxins in today’s spotlight. They are produced by different fungal species and occur in more than 40% of feed samples in varying concentrations (Figure 1). However, information about their effects on human and animal health is still scarce. Thus, information about the mode of action of emerging toxins is necessary. Cytotoxic effects were shown for the fungal metabolites beauvericin, enniatine (A, A1, B, and B1), bikaverin, and apicidin on a non-differentiated porcine intestinal cell line (IPEC-J2). IC₅₀ values were calculated and are close to the IC₅₀ value of the more prominent mycotoxin deoxynivalenol, which is already known to have detrimental effects on animals. As even low concentrations of emerging toxins might exhibit synergistic effects in combination with deoxynivalenol, combination studies with *in vitro* models are currently performed according to the method of Chou-Talalay (2010). First results indicate that some of the emerging toxins show synergistic or additive effect with the main mycotoxins. Furthermore, models will be established to study the counteracting effect of protective substances on different emerging toxins and deoxynivalenol.

![Figure 1](image-url)  
*Figure 1.* Occurrence of mycotoxins detected in > 40% of 814 samples analyzed by Spectrum 380® by BIOMIN in 2014.
P18: Epidemiology of Fusarium species producing enniatins in French wheat, durum wheat, barley and triticale

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Since 2006 (with EC Regulation 1881/2006) European legislation on contaminants is constantly changing, depending on the progress of scientific knowledge, mainly at the risk assessment carried out by independent agencies (European Food Safety Authority (EFSA) at EU level). In 2014, EFSA published a scientific opinion on beauvericin and enniatins. Indeed, enniatins are mycotoxins produced by various Fusarium species in the literature. In France, three of those Fusarium species occur and may produce enniatins: F. avenaceum, F. poae and F. tricinctum. Consequently, ARVALIS- Institut du végétal has conducted a field survey in collaboration with different French partners (mainly farmers, and FranceAgriMer). The study involved 1066 farm fields from 2012 to 2014. Wheat, durum wheat, barley and triticale were studied. For each field, a sample was taken at harvest and analyzed. Enniatins A, A1, B, B1 was analyzed by HPLC-MS/MS and F. avenaceum, F. poae and F. tricinctum were analyzed by qPCR. We first of all observed a correlation between the four enniatins A, A1, B, B1 leading to work on the sum of this 4 mycotoxins. On the other hand, a PCA realized on total Enniatins and individual Fusarium contents showed differences between each Fusarium species. A correlation is observed between enniatins, and both F. avenaceum and F. tricinctum. A statistical analysis was applied on ln(total enniatins). A variance analysis on data applied to harvest year, F. avenaceum, F. poae, F. tricinctum and crop showed that all these factors significantly influence enniatins content (p-value=2.2.10⁻¹⁶) and in a less extend for F. poae (p-value=0.017). A second statistical analysis on ln(total enniatins) by crop and by harvest year showed that F. tricinctum is the first contributor to enniatins content for wheat (20% to 43%), durum wheat (29% to 45%) and spring barley (23 to 37%). F. avenaceum is a significant contributor for triticale (30% to 55%), durum wheat (13% to 17%), wheat (1% to 18%). To finish, F. poae is a minor contributor for all crops, and always explains less than 10% of enniatins content. Enniatins content in small grain cereals depends first of all on the presence of F. tricinctum and F. avenaceum in grains. The contribution of each Fusarium in enniatins content depends on the crop susceptibility and the field weather conditions. Acknowledgements. This study was realized with the financial support of FranceAgriMer.

P19: Co-occurrence of fusariotoxins naturally produced by Fusarium species in French corn

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Corn is often infected by Fusarium species causing corn ear rot and producing mycotoxins, which may pose health risks to humans and animals. Deoxynivalenol (DON) and fumonisins (FB1-FB2) are the main Fusarium toxins identified in France. However, less is known about the co-occurrence of DON and fumonisins with other Fusarium toxins in France. This study examined the presence and the correlation of numerous Fusarium toxins in 262 corn samples representative of 2010 to 2014 harvests, collected in partnership with FranceAgriMer. Toxins levels were quantified with multitoxin analysis methods based on HPLC-MS/MS. The following fungal metabolites were analyzed: deoxynivalenol (DON), zearalenone (ZEN), nivalenol (NIV), diacetoxycescirpenol (DAS), T-2 and HT-2 toxins, fusarenone X (4-acetyl-NIV), fumonisin B1 (FB1), fumonisin B2 (FB2), beauvericin (BEA), enniatins A, A1, B, B1 (ENNAs), and moniliformin (MON). The presence of one toxin is considered positive since the toxin is above the analytical limit of quantification, which does not assume the sanitary quality of crops. It was found that toxins produced by F. graminearum: DON (100%) and ZEN (85%) were present in all samples. All samples contained one of the 3 following toxins: FB1-FB2 (58%), BEA (99%), MON (78%). ENNs were present in almost all samples (95%), T-2 and HT-2 toxins were present in 26% of samples (all containing ENNs). NIV was present in 31% of samples (all containing ENNs), and 17% of samples containing NIV contained also 4-acetyl-NIV. To finish, DAS were absent in all samples. Correlation studies confirmed the co-occurrence of DON and ZEN (r²=0.45) produced by F. graminearum. The co-occurrence of FB1-FB2 and MON was confirmed (r² = 0.69), as well as FB1-FB2 and BEA (r² = 0.72) and lastly BEA and MON (r² = 0.76). NIV co-occurred with and its metabolite 4-acetyl-NIV (r²=0.61). A PCA confirmed the correlations described above. These results showed that numerous Fusarium toxins co-occur in corn. This is the consequence of the co-occurrence or the quick succession of the related Fusarium species causing corn ear rot in France. Acknowledgements. This study was realized with the financial support of FranceAgriMer.
P20: Trichothecene mycotoxin levels detected in winter wheat in Ontario, Canada from 2009-2015

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Fusarium head blight caused by Fusarium graminearum is a serious disease of wheat (Triticum aestivum L). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated grain in Ontario. The objective of this study was to evaluate the level of trichothecene mycotoxins in winter wheat grain in Ontario from 2009, 2010, 2013, 2014 and 2015. The harvested grain was sampled to determine DON, 15-acetyl DON, 3-acetyl DON, nivalenol (NIV), T-2 and HT-2 toxins using a GC-MS system with a detection limit of 0.06, 0.05, 0.05, 0.12, 0.06 and 0.04 µg/g, respectively. In 2015, DON level was detected using ELISA method with a detection limit of 0.25 µg/g. The average DON level was 0.7 µg/g, 0.3 µg/g, 3.3 µg/g, 0.2 µg/g and 2.5 µg/g in 2009, 2010, 2013, 2014 and 2015, respectively. 15-acetyl DON and 3-acetyl DON were not detected in 2009, 2010 and 2014 in Ontario. However, they were detected in 2013 in soft white winter at one or two locations. NIV was not detected in any sample in 2009, 2010, and 2014 while was detected just in one sample in 2013 at level 0.14 µg/g. T-2 and HT-2 toxins were detected in one sample in 2009 at level 0.07 µg/g and 0.06 µg/g, respectively, while they were not detected in 2010 and 2014. In 2013, T-2 and HT-2 ranged from 0.08 µg/g to 0.14 µg/g and from 0.04 µg/g to 0.80 µg/g, respectively. In 2013, DON level was high in general, but lower mean levels of DON were detected in hard red wheat than in soft white wheat. DON level was low in 2014, and the highest detected level was in cv ‘Wentworth’ at Ridgetown location (1.6 µg/g). In 2015, averaged DON level was 2.5 µg/g, 5.9 µg/g, 2.0 µg/g, 0.9 µg/g, 5.0 µg/g at Inwood, Woodslee, Nairn, Palmerston and Ridgetown, respectively. In conclusion, several times higher average levels of DON were detected in 2013 and 2015 compared to previous years, with some winter wheat showing a level of tolerance to mycotoxins accumulation. Future monitoring of trichothecene mycotoxins in winter wheat in Ontario is recommend.

P21: Identification and characterization of Alternaria species causing early blight on potato in Belgium

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Alternaria species, including A. solani and A. alternata are a serious threat for potato cultivation since heavy infections can lead to significant yield and quality losses. Both species cause necrotic symptoms, which cannot be visually distinguished. Over the past years, both pathogens have become increasingly important in NW Europe. Although the exact cause for this emerging problem remains elusive, it might be attributed to the combined effect of climate change, a reduced use of the fungicide mancozeb, the increased specificity of active ingredients to control Phytophthora infestans and the production of high-yielding susceptible cultivars. Furthermore, little is known about the Belgian Alternaria population and the contribution of both A. solani and A. alternata to the disease. The main goal of this research is to identify the primary causal agent of potato early blight, to determine inter- and intraspecific diversity within the Alternaria population in Flanders, and to unravel the complex interaction between stress-related hormones and the Alternaria infection. To achieve these objectives, 124 fields were monitored throughout Flanders from 2013-2015. Results of this disease survey unequivocally show that the disease incidence and – pressure for all seasons was low and no significant differences between regions could be found. In a second part, we identified the population structure at the species level on different time points during the growing season. Therefore, leaf samples were collected during all three growing seasons and using a microscopic and molecular approach, we concluded that A. alternata, rather than A. solani, was the predominant species at the beginning of the growing season, while A. solani became apparent at later time points. Additionally, the genetic diversity of a subset of small-spore isolates was explored using a multilocus sequence typing analysis based on three conserved genetic regions. Our results show a high degree of diversity among the population and hints at a complex of different species, including A. tenuissima and A. arborescens. The same subset of isolates is now being tested for their pathogenicity and toxin fingerprint using high-throughput in vitro infection assays and an LC-MS/MS method, respectively. Based on the outcome of these analyses, a subset of isolates will be used to investigate the complex interaction between the disease progress and stress-related hormones such as ethylene or auxins. Indeed, previous research shows that ethylene is an important factor in Alternaria spore germination and that it is a key component in upstream signaling of programmed cell death induced by host-specific AAL-toxin.
HUMAN AND ANIMAL HEALTH
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P22: Is there a health risk concerning the children’s consumption of cereal-based products? A cumulative mycotoxin risk assessment approach

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Historically, the health risk of mycotoxins has been evaluated on the basis of single-chemical and single-exposure pathway scenarios. However, the co-contamination of foodstuffs with these compounds is being reported at an increasing rate and a multiple-exposure scenario for humans and vulnerable population groups as children is urgently needed. Cereals are among the first solid foods eaten by child and thus constitute an important food group of their diet. Few data are available relatively to early stages child’s exposure to mycotoxins through consumption of cereal-based foods. The present study aims to perform the cumulative risk assessment of mycotoxins present in a set of cereal-based foods including breakfast cereals (BC), processed cereal-based foods (PCBF) and biscuits (BT), consumed by children (1 to 3 years old, n=75) from Lisbon region, Portugal. Children food consumption and occurrence of 12 mycotoxins (aflatoxins, ochratoxin A, fumonisins and trichothecenes) in cereal-based foods were combined to estimate the mycotoxin daily intake, using deterministic and probabilistic approaches. Different strategies were used to treat the left censored data. For aflatoxins, as carcinogenic compounds, the margin of exposure (MoE) was calculated as a ratio of BMDL (benchmark dose lower confidence limit) and aflatoxin daily exposure. For the remaining mycotoxins, the output of exposure was compared to the dose reference values (TDI) in order to calculate the hazard quotients (HQ, ratio between exposure and a reference dose). The concentration addition (CA) concept was used for the cumulative risk assessment of multiple mycotoxins. The combined margin of exposure (MoET) and the hazard index (HI) were calculated for aflatoxins and the remaining mycotoxins, respectively. Main results revealed a significant health concern related to aflatoxins and especially aflatoxin M1 exposure according to the MoET and MoE values (below 10,000), respectively. HQ and HI values for the remaining mycotoxins were below 1, revealing a low concern from a public health point of view. These are the first results on cumulative risk assessment of multiple mycotoxins present in cereal-based foods consumed by children. Considering the present results, more research studies are needed to provide the governmental regulatory bodies with data to develop an approach that contemplate the human exposure and, particularly, children, to multiple mycotoxins in food. The last issue is particularly important considering the potential synergistic effects that could occur between mycotoxins and its potential impact on human and, mainly, children health. Acknowledgments. This research was performed under the MycoMix project ‘Exploring the toxic effects of mixtures of mycotoxins in infant food and potential health impact’ (PTDC/DTF-FTO/0417/2012) and CESAM Lisboa UID/AMB/50017/2013, both funded by the Fundação para a Ciência e Tecnologia (FCT), Portugal. The authors would like to thank Sonia Leal and her team (Primary Health Care Unit, Cidadela, Portugal), for all the support related to the consumption data collection; Sara Cunha and José Fernandes (LAQV-REQUIMTE, Portugal) and Alexandra Jager and Carlos Oliveira (University of São Paulo, Brazil), for the support on mycotoxin analysis.

P23: Assessment of mycotoxins in human milk of celiac mothers

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Gluten-free diet (GFD) is characterized by higher consumption of corn, rice and other cereals that may undergo to mycotoxin contamination. Among cereals, corn is the most susceptible to mycotoxins contamination. The presence of these contaminants on food products raises high concern because of their toxic effects such as genotoxicity, carcinogenicity, teratogenicity, immunosuppression both on animal models and humans. Celiac subjects under a GFD might be highly exposed to mycotoxins. The aim of this study was to assess the risk of mycotoxin exposure (aflatoxin M1, ochratoxin A and zearalenone) among celiac and control breastfeeding mothers (and their offsprings) by quantifying these contaminants in breast milk. Moreover, a nutritional survey on cereal consumption was run in mothers participating in the study, as well in a celiac and non-celiac nationwide cohort. During the study period
2011-2013, 35 celiac and 30 control breastfeeding women have been recruited. Human milk was collected throughout three days, during a complete 24 h period, as following: one milk sample in fasting, one 4 h after lunch and one 2 h after dinner. A total of 189 milk samples were collected. Mycotoxins content in breast milk were analyzed by immunoaffinity column cleanup and high-performance liquid chromatography with fluorimetric detection. As for the censored data analysis, a substitution method was applied following a lower bound approach. Dietary history on cereal consumption was recorded during the three days of breast milk collection. Aflatoxin M1 (AFM1) was detected in 37% of samples belonging to women with celiac disease (CD) (mean±SD =0.012±0.011 ng/ml; range=0.0035-0.340 ng/ml). Samples collected during fasting showed slightly higher concentration when compared to those collected 4 h after lunch and 2 h after dinner. When comparing to mothers with CD, the control group showed lower AFM1 concentration level in 22% of samples (mean 0.008±0.007 ng/ml; range=0.0035-0.0370 ng/ml). Estimating a daily average milk consumption of 530 g for a hypothetical body weight of 3.4 kg, the exposure of newborns from mother with CD and from control mothers resulted 1.87 and 1.24 ng/kg bw/day, respectively. No statistical significant difference was found as regards breast milk zearalenone content in both groups. Ochratoxin A was not significantly present in the investigated human milk samples of both groups. Co-occurrence of AFM1 and zearalenone was randomly found.

P24: Influence of cereal ergot on growth performance and digestibility in lambs

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This experiment evaluated the effect of different concentrations of cereal ergot on performance (experiment 1; n=48) and digestion (experiment 2; n=12) in weaned lambs. The experiment design was a two-way factorial (2 diet preparations × 3 alkaloid concentrations) resulting in six dietary treatments. Increasing concentration of ergot alkaloids (0, 150 and 350 μg/kg) in the diet was achieved by adding contaminated ground screenings. Diets contained approximately 54% barley grain, 30% alfalfa hay, 16% canola meal along with a trace amount of mineral and vitamin supplement and fed either as a mash or as a complete pelleted feed. The main alkaloids detected within the control diets (34±4.5 μg/kg) included ergocristine (~88%), ergocriptine (0.04%) and ergotamine (0.04%), indicating natural concentrations of ergot in the feed sample. In comparison, within both the 150 μg/kg and 350 μg/kg diets alkaloids included ergocristine (46.2±3.2%), ergocriptine (17±1%) and ergotamine (14±1.8%). In experiment 1, 48 weaned lambs (live weight (LW) 24.6±5.11 kg) were blocked by LW and randomly assigned to six dietary treatments and fed to a slaughter weight of ≥45 kg. Lambs were weighed weekly throughout the experiment to determine average daily gain (ADG). In Exp. 2, nutrient digestibility using 12 ram lambs was investigated using an incomplete two-way factorial 2×3 design with 3 experimental periods. All data were analyzed using the mixed procedure mixed of SAS with dose (0, 150 and 350 μg/kg), preparation (mash vs. pellet) and their interactions as fixed effects and lamb within treatment as a random effect. In experiment 1, feed intake was similar (p ≥ 0.53) across dietary treatments (1,345.2 ± 160.34). Lambs fed 350 μg/kg of alkaloids had lower ADG (p=0.03) compared to those fed control or 150 μg/kg. Lambs fed the pellet diet had a 19% greater (p<0.001) ADG compared to mash diet. In experiment 2, dry matter digestibility (71.58±0.948) was neither altered by diet form (p=0.16) nor alkaloid content (p=0.35). Neutral detergent fiber digestibility (45.44±1.76) was greater in lambs fed mash (p=0.005) as compared to pelleted diets. Pelleting increased ADG in all 3 doses, a response that appeared to be at least in part due to increased intake . Feeding ergot contaminated feed at concentrations of 350 μg/kg reduced ADG and therefore lowers feed conversion and economic output for the producer.

P25: Exposure to the mycotoxin zeralenone impairs embryo development in zebra fish

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A study was conducted to evaluate the developmental toxicity of three of the most commonly detected mycotoxins in food (deoxynivalenol (C15H2O6, mw=296.32 g/mol), patulin (C7H6O4, mw=154.12 g/mol), and zearalenone (C18H22O5, mw=318.36 g/mol)) using the zebrafish (Danio rerio) model. Mycotoxin exposures (0, 0.0064, 0.064, 0.64, 6.4 and 64 μM in 0.64% DMSO vehicle, n=32 dechorionated embryos/concentration for each mycotoxin) were initiated at 6 h post-fertilization (hpf) and were sustained until 120 hpf. We screened for delayed developmental progression, mortality and photo-induced tail flexion at 24 hpf; at 5 days post-fertilization (dpf), 18 different body morphology
endpoints and photomotor response were assessed. No significant developmental toxicity was associated with embryonic exposure to deoxynivalenol or patulin, except for modest hyperactivity for the highest dose of patulin (64 μM) in the 24 hpf photomotor assay. Significant mortality was associated with exposure to 64 μM zearalenone by 24 hpf, with additional mortality recorded in the 6.4 μM exposure group at 120 hpf. Exposure to 64 μM zearalenone was associated with significant incidences of adverse outcome in the majority of the 120 hpf body morphology endpoints. In addition, significant hypoactivity was associated with the 6.4 and 64 μM zearalenone groups in the 24 hpf photomotor assay. In summary, our results suggest that zearalenone may represent a developmental hazard, and that additional tests to further characterize the impacts of this mycotoxin on development and adult learning are warranted.

P26: Experimental study of deoxynivalenol biomarkers in urine

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Deoxynivalenol (DON) is one of the most commonly occurring trichotheccenes, produced mainly by Fusarium graminearum. The ESFA project ‘Experimental study of deoxynivalenol biomarkers in urine’ (GP/EFSA/CONTAM/2013/04, Question No EFSA-Q-2013-00717) aimed to provide data on levels of total DON and de-epoxy deoxynivalenol (DOM-1) in human urine samples collected from different population groups (children, adolescents, adults, elderly, vegetarians, pregnant women) in Italy, Norway and the UK as analyzed by liquid chromatography-mass spectrometry. Morning urine samples were collected over two consecutive days from 635 volunteers and associated food consumption was recorded on the same days. Levels of DON did not significantly differ between day 1 and day 2 urine samples. DON was detected in 99%, 93% and 76% of the urine samples from Norway, UK and Italy, respectively. The median total DON concentrations were similar between population groups in Italy and Norway, but were approximately 3-fold higher in the sampled UK population. In Norway and the UK, levels of DON were roughly 2.5-fold higher in children compared with adults. For DOM-1, 12 % of Norwegian and 1.5 % of Italian urine samples were positive, but DOM-1 was not detected in any sample from the UK. This difference may be explained by differences across analytical sites in the limit of quantification. Associations between food consumption and urinary DON levels were assessed by ordered logistic regression models. In Italy, intakes of pasta and pasta-like products were significantly associated with higher levels of total DON after correction for creatinine on both days. In Norway, intakes of breakfast cereals and snacks (day 1) and bread and bread-like foods (day 1 and 2) were significantly associated with a higher level of total DON adjusted for creatinine in both days. In Norway, intakes of breakfast cereals and snacks (day 1) and bread and bread-like foods (day 1 and 2) were significantly associated with a higher level of the toxin.

P27: Carry-over of aflatoxin B1 from dairy cows’ feed to milk

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Ingredients used in the cows diet, such as corn, can sometimes be heavily contaminated with aflatoxin B1. This mycotoxin was present in high amounts in corn from Italy in 203 and 2008, and corn grown in the Balkan in 2013. Aflatoxin B1 is metabolized and appears as aflatoxin M1 in dairy cows’ milk. Aflatoxin B1 and aflatoxin M1 are carcinogenic to animals and human. Hence, within the EC the presence of both toxins is regulated. Nowadays, milk production of dairy cows is increasing, as is the rate of corn used in compound feed production for dairy cows. High producing cows were indicated to have higher transfer rate of the mycotoxin. This may affect the appearance of and aflatoxin M1 in the cows’ milk. This study aimed to estimate concentrations of aflatoxin M1 in dairy cows’ milk, given contamination of compound feed with aflatoxin B1 and feeding consumption regimes. Monte Carlo simulation modelling was applied, with 100 iterations for each run. The model simulated two types of typical dairy herds of 100 cows. In the one scenario, all cows started their lactation at the same time, in the other scenarios the start of the lactations was spread over the years, with 2 cows starting each week. The composition of a typical compound feed for dairy cows was used. National monitoring data were used to determine the concentration range of aflatoxin B1 in each of the ingredients. In addition to this ‘standard’ contamination, we also used the concentrations of the toxin in corn as reported from the Balkan incident. Six different equations, from literature, were used to assess the transfer of aflatoxin B1 in the cow’s body from feed to milk. Modeling results showed that, in all scenarios considered, the maximum average
Aflatoxin M1 concentration in milk produced at the farm (all 100 cows) is below the EC legal limit of 0.05 μg/kg. In some scenarios, however, this maximum was close to this limit (up to 0.04 μg/kg). Apparently, the higher milk produced also dilutes the aflatoxin M1 in the milk.

P28: Transfer of aflatoxin B1 from feed to lambari fish (Astyanax altiparanae)

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Aflatoxins deposition in fish is known to be residual and cumulative, with effects mainly in muscle and liver. However, there are differences among species. Lambari fish (Astyanax altiparanae) has a production of approximately four hundred tons per year in Brazil. The aim of this study was to verify the transfer of aflatoxin B1 (AFB1) from feed to lambari fish. Aflatoxins were produced from Aspergillus parasiticus NRRL 2999 in culture material and incorporated to extruded feed previously tested. Following, the levels of toxins were checked by high performance liquid chromatography (HPLC). Treatments were constituted by: A, control - feed without toxin; B, feed + 10 μg/kg AFB1; C, feed + 20 μg/kg AFB1; and D, feed + 50 μg/kg AFB1. Fish were fed with 5% of animal biomass per day. Each treatment had 3 repetitions with 50 fish per m², adding up to 12 tanks. The unit sample was constituted by a pool of 10 fish. Aflatoxins B1 and M1 (AFM1) were quantified by HPLC in fish muscle and liver after 30 and 120 days of feeding. Means for AFB1 in fish muscle obtained after 30 days of feeding were 0.09 μg/kg, 0.29 μg/kg, 0.81 μg/kg and 1.27 μg/kg for treatments A, B, C and D, respectively. After 120 days, means in muscle were undetectable in control treatment and 16.67 μg/kg, 17.59 μg/kg and 47.66 μg/kg for treatments B, C and D, respectively. Levels of AFM1 in fish muscle were 0.13 μg/kg, 0.07 μg/kg, 0.89 μg/kg and 1.12 μg/kg after 30 days and 0.09 μg/kg, 0.23 μg/kg, 0.13 μg/kg and 1.15 μg/kg after 120 days for treatments A, B, C and D, respectively. Results of AFB1 in fish liver revealed levels of 0.05 μg/kg, 0.58 μg/kg, 0.88 μg/kg and 1.14 μg/kg for treatments A, B, C and D, respectively, after 30 days and levels below detection limit in control, 1.24 μg/kg, 264.87 μg/kg and 211.60 μg/kg for treatments B, C and D, respectively, after 120 days. AFM1 levels in fish liver were 0.37 μg/kg, 0.50 μg/kg, 1.63 μg/kg and 11.00 μg/kg after 30 days and 0.96 μg/kg, 1.31 μg/kg, 2.59 μg/kg and 2.77 μg/kg after 120 days for treatments A, B, C and D, respectively. It is concluded that AFB1 and AFM1 can accumulate in liver and muscle of lambari fish daily fed with contaminated diet, reaching concerning levels and representing a risk for consumer's health.

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P29: PR toxin toxicity on human cells – an in vitro study

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PR toxin is a mycotoxin produced by Penicillium roqueforti that can be found in grains and silage contaminated by this fungal species. It can also be detected in contaminated cheeses although not considered to be stable in the case of blue cheese, where this species plays an important technological role. This mycotoxin can be absorbed by humans or animals from contaminated foods and is considered to be the most toxic mycotoxin produced by P. roqueforti. PR toxin toxicity was already studied in rats and mice, however, there is a lack data related to humans. In this study, intestinal cell lines (Caco-2) representing the first barrier after ingestion and immune cells (THP-1) were exposed to different PR toxin concentration (1.25.10⁻⁵ to 1.25.10⁻⁶ M) for 48 h. Only 10% mortality was observed for Caco-2 cells for the highest tested concentration. For THP-1 cells, the IC₅₀ was determined to be 8.3.10⁻⁷ M after 48 h exposition and toxicity showed high necrosis (70% cell population) after 3 h exposition in the presence of 1.25.10⁻⁵ M PR toxin while at 1.25.10⁻⁶ M PR toxin, only 5% of cells were necrotic. Differential expression of 3 genes (TNFa, IL8 et IL-1β) coding for inflammatory cytokines involved in immune responses were studied and variations were observed after 6 h exposure to 6.25.10⁻⁷ M PR toxin in comparison to untreated cells. Highest expression levels were observed for TNFa after 24 h exposure. In conclusion, PR toxin is capable of activating the expression of 3 pro-inflammatory cytokines leading to inflammation and THP-1 cell necrosis.
P30: Carry over of deoxynivalenol and its 3-sulfate metabolite into eggs of laying hens

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Fusarium mycotoxins, like deoxynivalenol (DON), have been shown to adversely affect poultry health and productivity. The presence of mycotoxins in broiler feeds is a major concern for animal welfare and the development of more sensitive and specific analytical methods is resulting in new insights also on the carry-over into animal tissues and eggs. Recently, DON-3α-sulfate (DON-3-S) was reported as the main metabolite of DON in chickens. We developed an LC-ESI/MS/MS method based for the detection of DON, de-epoxy-DON (DOM-1) and their 3α-sulfates in freeze-dried eggs of laying hens after sample extraction with acetonitrile/water/acetic acid (79/20/1, v/v/v) and solid phase extraction (SPE) cleanup with phospholipid removal columns. Method performance characteristics like apparent recovery (RA), recovery of extraction (RE), matrix induced enhancement or suppression (SSE), limits of detection (LOD) and quantification (LOQ) were determined after spiking blank samples at multiple levels in triplicate. Average RA values ranged between 75 and 100%, RE from 80-112% and SSE from 90-99%. The calculated LOD ranged from 1.4 (DON-3-S) to 5.7 (DOM-1) µg/kg dried egg. The analytical method was applied to egg samples of a 10 week trial. 126 laying hens with an age of 17 weeks were assigned into the following 3 dietary treatment groups (42 hens/group, equally distributed into 14 pens: A, control; B, ~3-4 mg/kg DON; and C, ~7-8 mg/kg DON. In this approach, one egg of each pen was taken and 7 pools of two eggs each were prepared and lyophilized. Eggs were collected in the 5th, 7th, and 10th week of the animal trial. DON-3-S was the only metabolite detected. It occurred at levels of 17.1 ± 1.1 µg/kg, 22.7 ± 1.1 µg/kg, 25.1 ± 1.7 µg/kg, and 30.9 ± 4.9 µg/kg, 34.9 ± 2.5 µg/kg, 38.1 ± 3.3 µg/kg in samples from the dietary treatment groups B and C, respectively. In this study we report for the first time that DON-3-sulfate rather than DON itself is carried over into eggs. The additional trichothecone load appears to be of negligible concern for human health.

P31: Stability of masked mycotoxins under gastrointestinal conditions in vitro

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Fusarium mycotoxins are prone to metabolization and binding in the plant and their chemical structure is altered. These modified mycotoxins co-occur in contaminated cereal grains and plant materials. Their fate in the human gut and potential contribution to overall toxicity are not well understood. A large project funded by the UK Food Standards Agency aimed to address this. Cereal products, breakfast cereals, cereal-based infant foods, beers and spices on the UK market were analyzed for nine free and five modified trichothecone and zearaleneone mycotoxins. Deoxynivalenol (DON) and deoxynivalenol-3-glucoside (DON3Glc) and zearalenone (ZEN) were the major contaminants in cereal-based products and beers. Free DON and DON3Glc and ZEN were present in some breakfast cereal samples and in a few cereal based infant foods. ZEN was the most abundant mycotoxin in spices; bound forms of zearalenol (ZEL) and ZEN were present in some spices, as well as isolated occurrences of HT-2 and nivalenol. The metabolism and transport of glucoside metabolites of commonly detected trichothecones (DON, T-2 toxin) and zearalenone compounds (ZEN, α-ZEL, and β-ZEL) in the human gut were studied in vitro. Small intestinal metabolism was studied by incubating mycotoxins with artificial saliva, stomach juice, duodenal juice and bile. Intestinal transport of masked mycotoxins was assessed in fully differentiated Caco-2 clone TC7 cells. Large intestinal metabolism was studied using fecal batch cultures from 5 healthy donors. Mycotoxins and masked mycotoxins were analyzed using LC-MS/MS. All masked mycotoxins tested were stable under small intestinal conditions with no metabolism being observed. None of the masked mycotoxins were transported through the epithelial cell layer after 24 h. The large intestinal metabolism studies showed that masked trichothecones were hydrolyzed by human gut bacteria after 24-72 h whereas masked zearalenone compounds were hydrolyzed to parent compound within 4 h and further metabolized to unknown metabolites. Our results, using model systems, demonstrate that masked trichothecones will travel to the colon intact and could be potentially released as parent mycotoxins by gut bacteria, hence contributing to the overall mycotoxin exposure. The fate of masked ZEN compounds is different as both gut microbiota and gut epithelial cells (Cacao-
2 clone TC7) contribute to metabolism of these compounds and the nature and toxicity of ZEN metabolites warrants further investigation.

**P32: Fumonisin B1 and ochratoxin A and their biomarkers determination in animal tissues and serum as an assessment of dietary exposure**

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Assessment of human and animal exposure to mycotoxins is usually performed by the analysis of foods, feeds and beverages. However, a better method of assessing exposure to mycotoxins is to measure tissues and biological fluids. This approach is advantageous because it helps in estimating mycotoxins intake in terms of quantity and quality. The aim of this study is to determine the three major mycotoxins feed contaminants (fumonisin B1 and ochratoxin A), their tissues and serum biomarkers (sphingosine (So)/sphinganine (Sa)) as a way of assessing animal exposure. 203 samples (serum and tissues) were collected from pigs and analyzed using HPLC. The results obtained revealed that samples from rural and commercial farms were respectively contaminated with fumonisin FB1 in 85-87% of serum and 52-66% of tissues and ochratoxin A in 68-85% serum samples and 14-39% in tissues. Fumonisin B1 was found with a min 1.19 μg/kg in kidney samples followed by 0.69 μg/kg in liver and 3.4 μg/kg in muscle tissues while ochratoxin A was found at 28.5 μg/kg in kidneys, 15.5 μg/kg in liver and 1.8 μg/kg in tissue muscles. Analysis of So/Sa, which are the fumonisin biomarkers, revealed that there was no correlation between incidence of FBs in serum (87%) and tissue (60%) samples compared to So/Sa occurrence in serum (45%) and tissues (40%). In addition, among the samples analyzed (87%), the levels of FBs did not necessarily correspond to So/Sa in serum or tissues. This shows that there is no necessary association between FBs exposure and So (Sa) levels in tissue and serum as previously reported in some studies. It was also observed that not all fumonisin B1 positive serum or tissues samples had proportional So/Sa with higher So and lower Sa. The findings also contradict results obtained by Van der Westhuizen et al. (2008; 2010) who reported that there is correlation between fumonisin B1 exposure and So/Sa in monkeys. Fumonisin B1 is mostly found in muscle tissues mycotoxin while ochratoxin A was noticed in kidney mycotoxins. In conclusion, the novelty of this study is that it reveals the ability to detect more than one mycotoxin in analyzed samples but also raises issues of the effects of multi mycotoxins exposure for animals as well as humans consuming animal products. In addition, it confirms the fact that So/Sa are biomarkers for fumonisins but cannot be used for the quantification of fumonisins exposure in animals as previously thought.

**P33: Women and children exposure to mycotoxins: a case study of subsistence farmers in Shamva and Makoni Districts, Zimbabwe**

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Corn and legumes can be contaminated by mycotoxins if not properly handled during pre and post-harvest periods. Aflatoxin B1 and M1 are strongly associated with retarded growth in children and increased risk of contracting infectious disease. In this study, exposure of women and children under years of age was determined through analysis of fumonisin B1 and aflatoxin B1 in corn, levels of aflatoxin contamination in legumes they consume and aflatoxin M1 as a biomarker in their urine and breastmilk samples. Corn grain and meal were analyzed for fumonisin B1 using ELISA kits and aflatoxin B1 using high performance liquid chromatography coupled to a fluorescence detector. Aflatoxins B1, B2, G1 and G2 were determined in four legumes using immunoaffinity cleanup and high performance liquid chromatography. Aflatoxin M1 in urine and breastmilk was analyzed by immunoaffinity cleanup and analysis by high performance liquid chromatography. All the corn grain and meal samples from the districts were contaminated by fumonisin B1 ranging from 10.43-606.64 μg/kg and these levels are below the acceptable international regulatory limits of 2,000 μg/kg for FB1+FB2. However, on calculating the average probable daily intakes (APDI) of fumonisin B1 for the farmers in both districts, the APDIs were all above the provisional maximum tolerable daily intake of 2 μg/kg/per body weight/day. The calculated APDIs ranged from 3.13-5.68 μg/kg bw/day. This is because corn is the staple food in Zimbabwe and most rural people consume corn more than once a day. In corn samples, 20.6% (80/388) had fumonisin B1 and aflatoxin B1 co-occurring. Aflatoxin B1 levels in the corn samples ranged from 0.57-26.6 μg/kg and 23% of the samples exceeded the Zimbabwean set limit for aflatoxins of 5 μg/kg. Contamination of field samples of cowpeas, beans and round nuts with aflatoxins B1, B2, G1 and G2
were determined and total aflatoxins were calculated for each legume. Aflatoxins were detected in 10% (51/528) of the legume samples. Total aflatoxins detected ranged from 0.7 to 175.9 μg/kg. The Zimbabwean limit for total aflatoxins (15 μg/kg) was exceeded in 56.9% (29/51) of the aflatoxin positive legume samples. No breastmilk samples were found positive for AFM1. Urine samples, 4.6% (28/606) contained AFM1 with concentrations ranging from 0.37-51.49 μg/kg. The results showed that women and children from subsistence farming households are at high risk of exposure to both aflatoxin B1 and fumonisin B1 from the corn and legumes they consume.

P34: The effect of aflatoxins in the diet of weaned piglets on health and nutrient digestibility

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A study was set up to test the effect of 300 μg/kg aflatoxins on piglet health and nutrient digestibility. Thirty-six Duroc x Landrace x Yorkshire crossbred piglets (28-days-old) were randomly distributed over 2 dietary treatments. The study period was 36 days. The dietary treatments were: A, control diets with no measurable aflatoxins; and B, 300 μg/kg aflatoxins. Each treatment consisted of 3 animals per pen and 6 replicates per treatment. Body weight and feed intake were measured weekly. At the end of the study at day 36, one animal per pen was slaughtered and blood, feces as well as tissue samples were taken to analyze nutrient digestibility (via inclusion of a marker into the feed), blood chemistry, empty intestinal digestive enzyme activity, relative organ weights, intestinal morphology, intestinal mRNA expression of tight junction proteins and cecal microbiota. Aflatoxins reduced the final body weight and led to a tendency for a decrease in feed intake but had no effect on average daily gain or feed efficiency. No effect could be observed on nutrient digestibility. With respect to blood biochemistry, aflatoxins led to an increase in TNF-α. Other measured blood parameters seemed to be unaffected. Furthermore, aflatoxin had no effect on the activity of jejunal digestive enzymes and the jejunal mucosal morphology. Aflatoxins led to a decrease in relative heart weight and an increase in relative stomach weight. Tight junction gene expression was not influenced by aflatoxins, nor was the cecal microflora. It can be concluded that aflatoxins at a level of 300 μg/kg in diets reduced the body weight but only led to minor health effects on intestinal and digestive parameters in weaned pigs when fed for a period of 36 days.

P35: Incidence of fusariotoxin co-exposure on human monocytes

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Cereals are the most important food and feed resource in the world. Climatic and agricultural practices changes observed over the last years, including the reduction of fungicide use, could lead to food safety problems, especially concerning microbiological contaminations. Among microorganisms contaminating grains, some fungal species (Aspergillus, Fusarium and Penicillium) are toxigenic. In northern temperate regions of the world (America, Asia and Europe), Fusarium spp. are the most problematic species due to their prevalence, ecology, physiology and wide range of mycotoxins (called fusariotoxins) produced. Three fusariotoxin families are particularly important because of their high toxicity and their occurrence in European agricultural products: trichothecenes (mainly deoxynivalenol, nivalenol and T-2 toxin), fumonisins and zearalenone. The severity of the effects depends on the time of exposure, doses and mycotoxin combinations. Fusarium species are able to produce several mycotoxins simultaneously. Moreover, matrices can be simultaneously contaminated by different fungi species. Worldwide, 47% of the cereal samples analyzed in 2013 were multi-contaminated. However, the risk associated with this multi-contamination is no or a little studied for the moment, whereas there could have implications in various fields including regulatory. Indeed, to this date, mycotoxin regulation has been established for each mycotoxin considered individually. In this context, our main objectives were (1) to better characterize the risk induced by the simultaneous presence of Fusarium toxins in acute exposure conditions and (2) to study the cellular mechanisms involved in the response to the exposure to one or more mycotoxins through toxicology and proteomic approaches. For this purpose, we defined 4 fusariotoxin binary mixtures and we evaluated their in vitro toxicity on human monocytes THP-1. Effects on viability, mortality as well as expression of signaling pathway proteins such as MAP kinases (P38, SAPK/JNK, ERK1/2) were evaluated. After 48 h of exposure, IC50 for toxin T-2, nivalenol, deoxynivalenol and zearalenone were 0.007, 0.8, 2.2 and 35 μM, respectively. Only IC20 and IC30 were obtained at 10 μM for moniliformin and fumonisins B1, respectively. Co-exposure observed effects were mainly additives or antagonists. The study of cellular mechanisms involved in death showed early apoptotic and necrotic effects of mixtures (from 3 h of exposure). Finally, the study of signaling pathways...
involved in the cellular stress response to mycotoxin co-exposure demonstrated the activation of the phosphorylated ERK1/2 and P38 MAP kinases (from 1 h and up to 24 h) by low cytotoxic doses (IC_{50}).

P36: Do cyclic depsipeptide mycotoxins beauvericin and enniatins cross the blood-brain barrier?

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Cyclic depsipeptides beauvericin and enniatins are considered a potential health hazard, as for these emerging mycotoxins in vitro cytotoxicity and genotoxicity has already been evidenced. Besides absorption via inhalation or ingestion of contaminated food (Peitzsch et al., 2012; Devreese et al., 2014), it was recently demonstrated by our group that these mycotoxins are capable of reaching systemic circulation after dermal and mucosal exposure (Taevernier et al., 2014; 2015a,b). Since beauvericin and enniatins are thus able to reach the blood stream Devreese et al., 2014; Taevernier et al., 2015a,b) and neurotoxic effects have been demonstrated in vitro (Zhan et al., 2007), the general goal of this study was to investigate if these mycotoxins are able to pass the blood-brain barrier (BBB) and if so, to what extent. Prior to the BBB experiments, the metabolic stability of the mycotoxins was evaluated in vitro in mouse serum and brain homogenate. An in vivo mice model was used to study the initial BBB rate kinetics of beauvericin and enniatins. A blood-to-brain (multiple time regression influx) as well as a brain-to-blood (efflux) transport experiment were conducted. Additionally, capillary depletion was also investigated, giving information about the fraction transported into the brain (represented by the parenchyma) and the fraction trapped by the endothelial cells lining the BBB (represented by the capillaries). Blood was collected and brains were isolated at regular time points after administration to the anesthetized mice. Quantification of the mycotoxins was done using an in-house developed and basic validated bio-analytical UHPLC-MS/MS method (Taevernier et al., 2014; manuscript in preparation).

Metabolic stability data indicated that the investigated mycotoxins were stable during the duration of the study (85-115% recoveries). The influx study showed that beauvericin and enniatins are able to cross the blood-brain barrier in mice under our experimental conditions. Using a Gjedde-Patlak biphasic model, it was shown that all investigated mycotoxins exert a high initial influx rate into the brain (ranging from 11 to 53 μl/(g-min)), after which a plateau was reached during the second phase. No statistically significant efflux out of the brain was detected. On average 95% of the mycotoxins reached brain parenchyma after permeation of the BBB endothelial capillaries.

P37: Effects of Fusarium mycotoxins and feed restriction on broiler growth performance and immune response

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A 35-day feeding trial was conducted to evaluate the effect of Fusarium mycotoxins (primarily deoxynivalenol (DON)) and feed restriction on broiler growth performance and immune response. A 2*4 factorial arrangement in a completely randomized design with 8 replications was used to evaluate the effects of feeding programs (ad libitum vs. 75% restricted feeding) and mycotoxin levels (control, low, medium and high) on growth performance and immune response. A single source of naturally Fusarium damaged wheat was used to formulate experimental diets. The Fusarium damaged wheat were separated into high and low Fusarium damaged fractions based on individual kernel crude protein levels using a BoMill TriQ seed sorter. These two fractions of wheat were remixed at different ratios to achieve different mycotoxin levels. A total of 384 male Ross 308 broilers were raised in 64 cages and provided crumbled starter diets (3.0, 5.6, 9.0 and 13.3 mg/kg DON) from 1-21 days and pelleted grower diets (3.2, 6.1, 7.8 and 10.8 mg/kg DON) from 21-35 days. Birds’ growth performance was measured weekly. Injection of sheep red blood cells (SRBC) was used to evaluate immune response in control and high mycotoxin fed birds with primary antibody response measured on day 20 and secondary antibody response on day 27 and 34. Growth performance was not affected by dietary mycotoxins during the starter period; however, during the grower period, the broilers fed the ad libitum high mycotoxin diet consumed less feed (166 vs. 187 g/day) and gained less weight (100.7 vs. 113.7 g/day) than control birds. Restricted fed birds were lighter than ad lib fed birds throughout the experiment and dietary mycotoxin levels did not affect birds’ growth performance within restricted fed birds. Feed conversion ratio was not affected by dietary mycotoxins. This suggests that DON induced growth suppression in broilers is a result of reduction of feed intake. At day 34, high mycotoxin diet ad lib fed birds had higher secondary antibody responses than control and high mycotoxin diet restricted fed birds. Taken together,
dietary DON should be avoided from 21-35 days of age in order to achieve maximum growth. Interactions between feed restriction and dietary DON on chicken immune functions may require further study.

P38: Effects of feed-borne Fusarium mycotoxins on performance and intestinal histology in broiler chickens depends on timing of exposure

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In commercial practice, various sources of wheat are used as an energy source in broiler diets. Broiler might be exposed to Fusarium mycotoxins during a specific part or throughout the entire of production cycle, if Fusarium damaged wheat was used in feed production. A 34-day feeding trial was conducted to evaluate the effect of different Fusarium mycotoxins (primarily deoxynivalenol (DON)) exposure time on broiler growth performance and gastrointestinal structures. Five treatments (control, DON 1-14 days, DON 14-21 days, DON 21-34 days, and DON 1-34 days) were randomly assigned to 60 cages with 7 male Ross 308 birds/cage. Two mash starter diets and two mash grower diets were prepared with a clean wheat or a Fusarium damaged wheat. Control and DON diets were provided to broilers according to treatments, with starter diets (0.4 mg/kg and 6.6 mg/kg DON) from 1-21 days and grower diets (0.5 mg/kg and 7.9 mg/kg DON) from 21-34 days. Broiler growth performance was measured weekly. Segments of small intestine samples were collected on day 34 and morphometric indices were measured: villus height, crypt depth, villus width, thicknesses of submucosa and muscularis, and villus-to-crypt ratio. Birds fed DON starter diets over the first 14 days did not exhibit any changes in growth performance however, growth performance was suppressed in birds fed DON contaminated diets during grower period (21-34 days). At day 34, birds that received DON grower diet (DON 21-34 days and DON 1-34 days) were lighter (1,433 vs. 1,695 g) than control diet fed birds. Broilers fed DON grower diet had lower average daily weight gain during 21-28 days (60.5 vs. 73.6 g/day) and 28-34 days (74.5 vs. 96.5 g/day) than control diet fed birds. Feed conversion ratios were higher in birds fed the DON grower diet compared to controls during 21-28 days (1.77 vs. 1.56) and 28-34 days (2.24 vs. 1.85). Growth performance results suggest that providing older broiler chicks (21-34 days) feed contaminated with Fusarium mycotoxins (specifically DON) may result in greater loss to the producer. Histopathological analysis of the ileum region revealed that birds subjected to DON 1-34 days treatment had shorter villi (506 vs. 680 µm) and shallower crypt (85 vs. 115 µm) than control birds. Taken together, these results indicate that dietary DON-induced growth suppression may be a result of adverse effects on intestinal morphology during later growth phases of broiler.

P39: Aflatoxin exposure, gut function and child growth in Nepal

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Recent research indicates that exposure to aflatoxin, a type of mycotoxin produced by fungi that contaminate staple cereal crops, may affect child growth, and possibly through impaired gut function. Aflatoxin-related toxic damage to gut permeability, could increase susceptibility to infectious diseases such as those causing diarrhea, resulting in reduced nutrient bioavailability, potentially impairing child growth. The aim of the study was to investigate the relationship between aflatoxin exposure and gut function and its potential effect on child growth. Infants (n=77) participating in the MAL-ED birth cohort study from the Nepal site, an area of high incidence of undernutrition and diarrheal disease, were selected for the current analysis. Infants were assessed when aged 15, 24 and 36 months. At each visit length and weight measurements were collected along with blood samples for aflatoxin-albumin adduct (AF-alb) biomarker measurement. Vitamin A and zinc concentrations were measured in blood samples collected at 15 and 24 months of age. Indicators of child growth including length-to-age Z scores (LAZ), weight-to-age Z scores (WAZ) and weight-to-length Z scores (WLZ) were computed. To assess gut function, the following proteins were measured in stool samples collected at each visit: alpha-1-antitrypsin (ALA), myeloperoxidase (MPO) and neopterin (NEO). Geometric mean AF-alb concentrations at 15, 24 and 36 months of age were 3.85 pg/mg, (95%CI: 2.91, 5.09), 3.09 pg/mg (95%CI: 2.42, 3.94) and 4.04 pg/mg (95%CI: 2.94, 5.54). Linear regression analysis showed that aflatoxin exposure was not associated with change in LAZ/WAZ/WLZ scores from 15 to 36 months of age. At 15 months of age, aflatoxin exposure was inversely associated with MPO (β=-0.21, SE=0.08; p=0.012). A pathway analysis revealed that LAZ scores were not associated with MPO, NEO, ALA, AF-alb, vitamin A, zinc and anemia at 15 or 24 months of age. In a multilevel analysis of all available data,
no correlation was found between aflatoxin and child growth. Results from the current study did not support the hypothesis that aflatoxin exposure affects child growth through impaired gut function. The small sample size and the low aflatoxin concentrations observed, however, may have prohibited this relationship being adequately assessed. Further studies should investigate this relationship in regions where aflatoxin exposure, impaired child growth and environmental enteropathy are highly prevalent.

SAMPLING & ANALYSIS
P40 – P79

P40: Determination of 25 mycotoxins in Western Canadian oats from 2014-2015 crop years using LC-ESI/MS/MS with fast polarity switching and scheduled MRM

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This study of Western Canadian oats for the presence of mycotoxins over the last two years was done in order to help identify and manage the risks from the presence of these toxins. The 25 mycotoxins analyzed include the regulated one’s such as ochratoxin A (OTA) and deoxynivalenol (DON) as well as some non-regulated mycotoxins, such as Alternaria toxins, enniatins and beauvericin. Samples submitted for testing were ground on a Retsch Sr 300 mill using a 500 μm screen. Samples were then divided on a Materials Sampling Solutions rotary sample divider. Subsamples were then extracted along with normal, fortified and certified reference samples. The analytical method uses a simple solvent extraction followed by further dilution and injection of sample extracts on a Waters UPLC, integrated with an AB Sciex Triple Quad 5500. Of the 25 mycotoxins analyzed, 17 compounds were detected in the positive ionization mode and 8 were ionized in the negative mode. Quantitation uses both C13 internal and matrix matched standards. This method has been validated for cereal crops (wheat, oats, barley and corn) and over the past three years the method has demonstrated acceptable results for numerous proficiency testing programs (FAPAS, MoniQA, Coda-Cerva and Neogen). There have been ~300 oat samples analyzed to date and still have one more year left in this study. A summary of the analytical data generated of these mycotoxins analyzed for will be presented. The Grain Research Laboratory of the Canadian Grain Commission has an extensive grain safety assurance program in place to address food safety issues affecting grain. This program includes the analysis of pesticide residues, mycotoxins and trace elements in grains.

P41: The effect of grind size and extraction size on fumonisin result variability

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Fumonisin contamination in the U.S. corn crop can be erratic. Sample preparation of products being tested for fumonisin is a critical part of the total analytical process. It is well documented that sampling contributes a large portion of the total overall analytical variability. Differences in sample grind size as well as the amount of sample extracted can also contribute to the overall analytical variability. An evaluation was conducted to compare the extraction study of corn naturally contaminated with fumonisin utilizing different sample grinds and different sample extraction weights. The naturally contaminated corn was ground to various mesh sizes, homogenized and various sample sizes were extracted. Extractions were done with methanol/water (3:1). The extracts were then analyzed by HPLC using a modification of AOAC method 995.15. Data presented shows the effect grind size and sample extraction size has on the result variability.

P42: A new look at citrinin: quantitative analysis by HPLC and LC-MS/MS utilizing immunoaffinity purification

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Citrinin, a naturally occurring toxin produced by Penicillium, Monascus, and Aspergillus species of fungus, is most commonly considered a nephrotoxic compound. It is a mycotoxin that typically occurs in the storage processes of grains such as corn, soybeans, wheat, barley, and rice (red yeast rice). Evaluations were conducted through an R-Biopharm Rhône immunoaffinity column. This method allowed a single-extraction validation of both HPLC and LC-MS/MS with acceptable recoveries. This analysis uses the same purified extract to be injected onto the HPLC and/or LC-MS/MS. Use of this
method has shown limits of quantification at 10 µg/kg for several different matrices giving a better look at detection of this toxin.

P43: The adsorption and desorption capabilities of an aluminum silicate analysis containing multiple mycotoxins

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Aluminum silicates are commonly used as additives in animal feeds to minimize the absorption of mycotoxins by the animal. Typical analysis of these aluminum silicates check for single mycotoxins. An evaluation was conducted to determine if multiple mycotoxins could be analyzed with results comparable to single analysis. Allatoxin B1, deoxynivalenol, fumonisin B1, ochratoxin A, T-2 toxin and zearalenone were prepared in buffer solution and added to a recommended dosage of a previously analyzed aluminum silicate. The buffered solutions were then analyzed for adsorption and desorption using pH 3 for adsorption and pH 6.5 for desorption. Results proved that the analysis of multiple mycotoxins could be performed with comparable results to single analysis under the same conditions.

P44: Development of a multiplex mycotoxin microarray

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Mycotoxins are a group of naturally occurring toxic secondary metabolites produced by organisms of the fungi kingdom. They grow on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits and coffee, under warm and humid conditions. Immunological assays have shown great promise as sensitive screening tools while multiplex assays enable the detection of multiple targets in one single test. A highly sensitive and novel multiplex assay was developed for the semi-quantitative and simultaneous screening of three key mycotoxins (zearalenone, T-2 toxin and fumonisins). Individual antibodies and mycotoxin conjugates specific to the three mycotoxins were spotted onto wells of a microtiter plate and a competitive assay format was employed using two assay formats. This work has demonstrated the potential of multiplex analysis for mycotoxins with results available for 40 samples in as little as 60 min (direct assay) or 70 min (indirect assay) for three key mycotoxins. The characteristics and performance of the assay was evaluated for feasibility as a screening tool for mycotoxin determination in order to replace the traditional ELISA. Optimum parameters and antibodies were selected and both single (n=1) and multi (n=3) calibration curves were examined as well as precision (n=3) for both assay formats. Sensitivity was assessed by examining the IC50 of the assay with higher sensitivities achieved for all three mycotoxins using the direct assay. IC50 of 6.97 ng/ml (fumonisins), 0.26 ng/ml (T-2 toxin) and 1.02 ng/ml (zearalenone) were obtained in buffer for the multiplex direct assay format. In comparison, IC50 of 61.70 ng/ml (fumonisins), 2.04 ng/ml (T-2 toxin) and 41.28 ng/ml (zearalenone) were obtained in buffer for the multiplex indirect assay format. This method, therefore, has the potential to be used as a fit for purpose screening technique for mycotoxins within the area of toxin determination and food safety.

P45: Functions of a testing laboratory for mycotoxins and endophyte toxins

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The Oregon State University Colleges of Veterinary Medicine and Agricultural Sciences instituted the Endophyte Service Laboratory to aid in diagnosing the toxicity problems associated with cool-season grasses in livestock. Samples of feed material are sent to the laboratory for quantitative analysis of the mycotoxins. The laboratory services a large straw export industry to certify that the straw sent to Japan and the Middle East is below a toxic level for the animals that will ingest it. In addition, many veterinarians throughout the country will send in samples as a rule in or out for the diseases that they are seeing. Three toxins are of especial interest and they include the following: the endophyte (Neotyphodium coenophalum) present in tall fescue (Festuca arundinacea) produces ergopeptine alkaloids, of which ergovaline is the molecule used to determine exposure and toxicity thresholds for the vasoconstrictive conditions ‘fescue foot’ and ‘summer slump’. A second vasoconstrictive syndrome, ‘ergotism’, is caused by a parasitic fungus Claviceps purpurea and its primary toxin, ergotamine. This toxin has been increasingly seen in the Pacific Northwest in part due to the cessation of field burning of grass straw and the use of straw as fiber for animals. Thirdly, ‘ryegrass staggers’ is a neurological condition that
affects livestock consuming endophyte (Neotyphodium lolii)-infected perennial ryegrass (Lolium perenne) with high levels of lolitrem B. HPLC-fluorescent analytical methods for these mycotoxins are described and illustrated. Studies were undertaken utilizing feeding trials to determine threshold levels of toxicity for ergovaline and lolitrem B in cattle, sheep, horses, and camels.

P46: Multimycotoxin LC-MS/MS screening in feed

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Among mycotoxins present in feed, there is a ‘gold’ group regulated by the European Commission (Directive 2002/32/EC, Recommendation 2006/576/EC): aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2, T-2 and HT-2 toxins. They are secondary fungal metabolites with different acute and chronic toxicity. They are representative of the common fungal flora present in feed. All these may affect either the animal health and the productivity. Nonetheless some of these mycotoxins as well as their metabolites can carry over the animal origin food, for instance aflatoxin M1 in milk and dairy product. This is the reason why it is very important to have an analytical method able to quickly detect all these regulated mycotoxins. In this work we developed a very fast screening of the above mentioned mycotoxins. The method is based on a ‘dilute & shoot approach’: a single solvent extraction was followed by a proper dilution and LC-MS/MS injection. In a single run of 11 min the separation of the analytes occurred. The method was validated on 29 FAPAS reference materials ranging from simple feed, such as corn flour or barley flour, to compound feed. The QC materials were either mono- or multi-residue assigned. Experimental z-score values were all found in the satisfactory range, i.e. 87% in the range of ±1 and 13% in the range ±2. The high-throughput performances of the method can be really considered suitable for routine controls, mainly whenever a great number of samples have to be analyzed.

P47: Determination of ochratoxin A in meat, meat products and edible offal: preparing the intervalidation study for CEN standard method

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The European Commission invited CEN to establish European Standards or Technical Specifications to provide standardized methods of analysis for mycotoxins in food. For this purpose, a mandate (N520 of 6 March 2013) was published and the item 8 (Determination of ochratoxin A in meat, meat products and edible offal) was assigned to the Italian National Reference Laboratory (NRL) for mycotoxins in food and feed, held by the GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety Italian National Institute for Health. Ochratoxin A (OTA) contamination may occur indirectly by a carry-over from feed to edible products of animal origin or directly during the wet curing process of some kind of pork based products, namely Parma-like ham. Despite the absence of a Community maximum level, the Italian Ministry of Health set a guideline value at 1.0 µg/kg in pork meat and derived products since 1999 on the basis of the precautionary principle. For this purpose, the NRL set up a method for the analysis of cured ham and performed a single laboratory validation study. This study was accepted for the method validation study (MVS) provided that field of application and applicability to different matrices were adjusted. Therefore, the preparation of the MVS was preceded by the single laboratory validation on a higher level of contamination of cured ham and the validation of OTA analysis in liver and other pork-based products (namely pâté and minced pork). Moreover, it was verified the performance of different brands of immunoaffinity columns. Only after checking the availability of enough contaminated material of suitable matrices, and after programming the homogeneity and stability checks, the announcement of the study was launched. Three levels of cured ham, a liver sample and a minced pork based product have been characterized as materials for the MVS. The performances of the single laboratory validation and the homogeneity and stability tests are presented. The MVS started in May 2016 with the participation of 25 laboratories from up to 9 different EU countries. The results reported back by the participants will be processed and embedded to produce the CEN standard.
This work aimed at assessing two processes of sampling grains stored in silos for analyses of mycotoxins and determination of the nutritional composition. Samples were collected from four corn silos, located in Rio Grande do Sul state, according to two sampling procedures (A and B). Samples collection was performed in triplicate, through the introduction of a pneumatic probe into the middle and in the four central points of each quadrant from the surface to the bottom of the silo. In sampling procedure A, grains collection was divided in three 4 kg samples in each sampling point: upper, middle and lower third of the silo. In sampling procedure B, only one sample of 12 kg was collected in each sampling point, consisting of grains taken from the entire vertical profile of the silo. The samples were then ground through 2 mm screen sieve and reduced to 500 g by using a Jones-type sample splitter. The samples were analyzed for the presence of aflatoxins, fumonisins and zearalenone in a NBR ISO 17025 accredited laboratory by LC-MS/MS. Prediction of the values of dry matter (DM), crude protein (CP), ether extract (EE) and apparent metabolizable energy for poultry corrected for nitrogen balance (AMEn) employed near infrared reflectance spectroscopy (NIRS). No significant difference was found via the F test (p>0.05) between the two sampling procedures (A and B) in the coefficients of variation (CV), calculated by sampling point, for aflatoxins (54.9 and 66.4%), fumonisins (19.4 and 27.4%) and zearalenone (68.5 and 85.5%). Moreover, there was no effect (p<0.05) of the covariates silo and contamination for aflatoxins, fumonisins and zearalenone. Although having a small variation between the two sampling procedures (A and B) in each studied variable, the nutritional composition presented significant difference through the F test (p<0.05) for the CV of: CP (1.1 and 1.8%), EE (0.4 and 0.9%) and AMEn (0.2 and 0.4%). Despite the greater economic viability of sampling procedure B, both methods of sampling grains stored in silos provide representative samples for the determination of mycotoxin contamination and nutritional composition. Thus, both procedures allow for prior planning and proper allocation of stored grains, being reliable management tools regarding the risks posed by mycotoxins and determination of the nutritional composition of the stored corn.

It is a worldwide consensus that a result is not complete without an expression of its uncertainty and its estimation is a requirement for testing laboratories accreditation by the International Organization for Standardization (ISO). The uncertainties of aflatoxins (B1, B2, G1, G2) concentration in corn, determined by liquid chromatography tandem mass spectrometry, were estimated using the cause and effect approach proposed by ISO GUM (Guide to the Expression of Uncertainty in Measurement) following its main four steps. The relationship between the measurand and the input quantities it was dependent on was stated. The measurand was defined as the concentration of each aflatoxin in a corn sample. The data required to produce an estimate of measurement uncertainty was obtained from a precision study by carrying out 21 analyses of different corn samples free of aflatoxins contamination (blank samples) and fortified with aflatoxin B1, B2, G1, and G2 at 1.0 µg/kg each. The sources of uncertainties due to volume measurements, calibration curve, allowed range for recovery variation and precision were taken into account in the uncertainty budget. For volume measurements the sources of uncertainties due to calibration, resolution, laboratory temperature variation and repeatability were also considered. The uncertainty of the quantification arising from the calibration curve was obtained by weighted least square regression. The standard uncertainties arising from each source were estimated by considering type A and type B evaluations. The calculation of the result and its associated standard uncertainty was performed by applying the model and combining the contributions into the standard uncertainty of the result. The combined uncertainty was obtained as the positive square root of the sum of the relative variances from the following steps: preparation, extraction, dilution, calibration curve and precision. The mean aflatoxins concentrations and the respective expanded uncertainties were aflatoxin B1 0.90 µg/kg ± 0.15 (k=2.12), B2 0.91 µg/kg ± 0.15 (k=2.12), G1 0.92 µg/kg ± 0.22 (k=2.13) and G2 0.85 µg/kg ± 0.21 (k=2.13) with approximately 95.45% of coverage probability. The main sources of uncertainties in aflatoxins quantification were the precision and calibration curve (corresponding to more
than 70.0% of the global uncertainty). On the other hand, the uncertainty components from weighing and volume measuring were small. The modeling approach proposed by ISO GUM provided a detailed and consistent manner to estimate the combined uncertainty of the aflatoxins in corn by liquid chromatography tandem mass spectrometry.

P50: Validation of a method for the analysis of citrinin in cereals using immunoaffinity columns

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Citrinin is produced by a number of Aspergillus and Penicillium fungi and has been found in a variety of foods, such as grains, cheese and red yeast rice, the latter of which is regulated in the EU (Commission Regulation (EU) No 212/2014). Although legislation is currently only in place for red yeast rice, there is considerable interest in Europe with regards to levels in foods including cereals, as this toxin is considered as a potential issue during storage often occurring simultaneously with ochratoxin A with both toxins considered as potential agents of Balkan endemic nephropathy. R-Biopharm Rhône have developed a new immunoaffinity column which selectively isolates and concentrates citrinin from a wide range of commodities including cereals and red yeast rice. A simple extraction with 75% methanol was used to analyze a range of cereal samples spiked at 100 μg/kg. Average recoveries ranged from 74% for multigrain cereal to 96% for oat groats demonstrating that the new EASI-EXTRACT® CITRININ columns were suitable for the cleanup of the toxin from a wide variety of cereal samples resulting in improved chromatography and lower limits of detection.

P51: Multi-toxin analysis using immunoaffinity column clean-up for a range of samples prior to LC-MS/MS detection

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Frequently in mycotoxin analysis there is a need to analyse for combinations of mycotoxins as dictated by the susceptibility of the commodity to specific fungal infection and the regulatory limits which apply. Most official methods, which have been rigorously validated, stipulate the use of immunoaffinity column cleanup prior to HPLC analysis. R-Biopharm Rhône’s immunoaffinity columns offer a versatile solution for multi mycotoxin analysis whereby the immunoaffinity columns can be used in tandem with one another to cover the regulated mycotoxins applicable to a particular food matrix. AOF MS-PREP® and DZT MS-PREP® immunoaffinity columns were tested in tandem to determine the applicable mycotoxins (total aflatoxin, ochratoxin A, fumonisin, deoxynivalenol, zearalenone, T-2 and HT-2) in a number of cereals and cereal based products. The samples were analysed using a single extraction followed by immunoaffinity cleanup with the columns connected in tandem prior to LC-MS/MS detection. Matrices analysed were corn-based infant food, beer, bread and breakfast cereal. When testing the various samples using the tandem method results met EU Method Performance Criteria in terms of recovery and % RSDr. This study shows that a single extraction with immunoaffinity column cleanup can be utilised for the analysis of 11 mycotoxins in one LC-MS/MS run. This approach is not only cost effective but offers a greater flexibility of testing options to customers analysing for a wide range of mycotoxins.

P52: Determination of six major ergot alkaloids and their epimers in cereal grains using LC-MS/MS

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Ergot alkaloids are mycotoxins that are produced by the Claviceps purpurea fungus. This fungus grows parasitically on grasses and cereal crops throughout the world. The fungal infections occur during the flowering stage where the fungus replaces the grain or seed with the alkaloid containing purplish body known as sclerotia or ergot body. These alkaloids are toxic to humans and animals and ergotism is the term used to describe the condition after eating food or feed contaminated with ergot. Due to the toxicity of these alkaloids there are strict tolerances in crops which can result in economic losses by rejection of grain or downgrading at the elevator. This method analyzes for the six major ergot alkaloids and corresponding epimers. The method involves the extraction of ground sample with 84/16 (v/v) acetonitrile/3 mM ammonium carbonate. The samples are centrifuged, a further 1/4 dilution with 3mM ammonium carbonate and filtered through PTFE filters into autosampler vials. The analysis is on a Waters LC-MS/MS consisting of an Acquity UPLC and Xevo TQ. The separation is performed on a
Waters BEH C18 1.7μm 2.1 x 100 mm column using a gradient elution with A1: 3mM ammonium carbonate and B1: acetonitrile. The Xevo TQ is operated in the ESI+ mode and the MRM method consisted of two transitions for each ergot alkaloids and epimers and one for the internal standard dihydroergotamine. The six ergot alkaloids analyzed for are: ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, and ergocristine and their respective epimers. The LOQ for all of the analytes is 10 μg/kg, except for ergonovine which is 2 μg/kg. The method has been validated using an in-house procedure to characterize the accuracy, repeatability, and intermediate reproducibility of the analysis in wheat and rye samples. The method has been involved in 2 FAPAS PT studies with acceptable results. A summary of the data generated over the past few years will be presented. The Grain Research Laboratory of the Canadian Grain Commission has an extensive grain safety assurance program in place to address food safety issues affecting grain. This program includes the analysis of pesticide residues, mycotoxins and trace elements in grains.

P53: Accurate lateral flow aflatoxin testing with environmental-friendly aqueous extraction

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Organic solvents always played an important role in mycotoxin analysis. However, they have a negative impact on the environment and impose a hazard for the lab technician. Research showed that alternative, less toxic, organic solvents and even aqueous solutions can be used for the extraction of mycotoxins. Aqueous solutions are also more cost effective. Some mycotoxins easily dissolve in water, while aflatoxins do not. Like many other mycotoxins aflatoxins prefer organic solvents. In 2008 a method was published in the journal of the AOAC using water with a detergent (SDS) as an additive. It was shown that the aqueous extraction of aflatoxin from corn was effective. Since SDS is not very environmental-friendly R-Biopharm set out to develop a lateral flow test with an eco-friendly aflatoxin extraction for rapid and precise testing. Here we present data showing the accuracy and precision of the total aflatoxin lateral flow test RIDA®QUICK Aflatoxin RQS ECO. The extraction of samples was performed with an eco-friendly aqueous solution. Without further dilution the extracts were tested with the lateral flow test strips. Presented results were generated by analyzing Trilogy® corn reference material naturally contaminated with aflatoxin. Reference materials were analyzed by the reference material manufacturer by HPLC using AOAC method 994.08 with modifications. 14 Trilogy® corn reference materials were extracted 3 times each and analyzed with RIDA®QUICK Aflatoxin RQS ECO. Two reference materials were found to be below the limit of detection of the RIDA®QUICK Aflatoxin RQS ECO (<4 μg/kg), two showed results above the upper end of the detection range (>75 μg/kg), both in accordance with reference material certificates. 10 samples were determined to be positive within the detection range with a very good mean recovery of 95%.

P54: Analysis for Fusarium mycotoxins in single kernels of wheat

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Fusarium is a fungus that is prevalent around the world and can adversely affect the quality of small grains used in milling, baking and brewing and can also produce toxic metabolites known as mycotoxins which are a concern to animal and human health. Species of Fusarium that are prevalent in Canada are Fusarium graminearum, F. culmorum, F. avenaceum, F. sporotrichioides and F. poae. Deoxynivalenol (DON) is the major mycotoxin produced by Fusarium in Canada. The kernels that are affected by fusarium are known as fusarium damaged kernels (FDK). An investigation looking at the types and levels of mycotoxins that can be produced in single kernels of wheat was undertaken. The study looked at different levels of fusarium infestation on the kernels. Grain Inspectors at the Canadian Grain Commission categorized the kernels as sound, mild (infection) and severe (infection). Several different classes of wheat were also investigated namely, Canadian Western Red Spring (CWRS), Canadian Western Amber Durum (CWAD), Canadian Western Red Winter (CWRW), Canadian Eastern Soft Red Winter (CESRW), Canadian Eastern Hard Red Winter (CEHRW), Canadian Eastern White Winter (CEWW) and Canadian Eastern Red Spring (CERS). Fusarium mycotoxins DON, nivalenol, 3ADON, 15ADON, fusarenon-X, diacetoxyscirpenol, HT-2 and T-2 were analyzed in 1,036 individual wheat kernels by a modified gas chromatography with mass selective detection method. 97.7% (n=340) of all the visually sound kernels contained no DON while 2.3% (n=8) contained measurable amounts of DON. 54.6% (n=190) of all the visually mildly infected kernels contained no DON while 45.4% (n=158) contained measurable amounts of DON. 20.3% (n=69) of all the visually severely infected kernels contained no DON while 79.7% (n=271) contained measurable amounts of DON.
P55: Optimal sampling and extraction procedures for ergot measurements

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Ergot, a mycotoxin produced by the fungus Claviceps purpurea is a contaminant of cereal crops and grasses. Consumption of ergot alkaloids is toxic to humans and domestic animals causing a wide range of vascular, thermoregulatory, endocrine and neurologic disorders. Three methods have been used to report ergot concentrations in grain and feed: (1) count (number of ergot particles); (2) weight (weight of ergot particles); and (3) analytical (total alkaloid concentration). Current Canadian food and feed safety guidelines allow human food and livestock feed grain to contain 0.01-0.05% and 0.10% net ergot weight, respectively. Variation at low concentrations and different methods of analysis can lead to unreliable estimates of the ergot concentration which can result in human or animal exposure to toxic amounts. This study was conducted to evaluate the variability of three methods (counting, weighing and ergot alkaloid measurement after extraction) at different sample volumes and concentrations. This study also assessed the variability attributable to grinding method during the extraction and liquid chromatography-mass spectrometry (LC-MS) analysis. For analyzing variability among methods, five concentrations (very high to very low) and four sample volumes (75, 250, 500 and 1000 ml) were tested. For each sample, the number of ergot particles were counted, weighed and recombined for grinding and extraction using a solvent mixture (85% acetonitrile/15% 10mM ammonium acetate) and measured via LC-MS. Variability introduced by the grinding procedure was examined by adding one ergot body of uniform weight (0.0148 ± 0.0002 g) to 80 g of wheat and grinding each sample using either a coffee grinder or sample mill grinder (5 replicates each). All samples were extracted then measured via LC-MS. For the analytical method, coefficient of variation was reduced as sample volume increased (98% CV for 75 ml versus 64% CV for 1000 ml) but increased as sample concentration declined (17% CV for very high; 284% CV for very low). Similar trends were seen for the other two methods. The coffee grinder resulted in a 57% higher coefficient of variation in total alkaloid concentration compared to the sample mill and a difference in total alkaloid concentration was seen between the ergot bodies of the same weight during the grinder comparison. To conclude, analytical measurement is considered to be the best detection method when used with the appropriate grinding method to produce consistent and reliable analysis.

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P56: The use of laboratory automation in the routine analysis of EU-regulated mycotoxins by UHPLC-MS/MS

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With the increased usage of ultra high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry, the analysis of mycotoxins has been greatly simplified and improved. Lower detection limits, reduction of false positives and confirmation of mycotoxin identity by mass spectral data are examples of the superiority of this technology compared to traditional means of detection. Bottlenecks in previous analytical procedures, such as immuno affinity column purification, solid phase extraction, liquid-liquid partitions, and derivatizations, have largely been eliminated for the vast majority of samples. Analytical throughput has also been dramatically increased with the use of small particle UHPLC columns as compared to traditional high performance LC columns. Where several extraction and detection methods were once a necessity for the analysis of different classes of mycotoxins, a single extraction and injection are now commonly employed. As these technological advances are implemented, new bottlenecks have been identified. Routine liquid handling, such as sample dilution, internal standard addition, sample filtration, and calibration standard preparation can be time consuming for the bench chemist. Also, the documentation requirements in an ISO 17025 facility can consume a significant amount of time for laboratory personnel. This poster outlines automation and data collection processes put in place that allow for the analysis of the EU-regulated mycotoxins in over 100 samples daily by a staff of two chemists.
P57: Evaluating the BGY fluorescence signal in corn kernels inoculated with various aflatoxin producing fungi using fluorescence hyperspectral imaging

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Recent studies applied fluorescence hyperspectral imaging to non-invasively screen aflatoxin-contaminated corn based on BGY fluorescence exhibited by kernels inoculated with a specific aflatoxin producing Aspergillus flavus fungal isolate, AF 13. The current experiment utilized the same imaging method to determine if kernels exhibit similar fluorescence when inoculated with other aflatoxin producing Aspergillus isolates. Several different aflatoxin producing fungal strains, including different strains of A. flavus and A. parasiticus, were obtained from the SRRC-ARS-USDA culture library and cultured on PDA. Conidia were harvested after 5 days and inocula were produced from each strain. Thirty kernels per strain (24 treatment and 6 control) were surface sterilized in 70% ethanol followed by 3 rinses with distilled water and one-minute inoculum treatment. Inoculated kernels were placed into plastic incubation trays. Eight treatment reps of 3 kernels/rep plus 2 control reps were included in each tray. Trays were flooded with distilled water and incubated for 5-7 days at 30 °C. Length of incubation was determined by rate of colonization of individual isolates. Fungus-covered kernels were imaged on a clay imaging tray, one image of 30 kernels per strain. Kernels were imaged under both UV illumination and halogen lights with mold growth and wiped clean, on both germ and endosperm sides resulting with 6 images per strain. Imaged kernels were dried and processed for aflatoxin determination. Images are being evaluated for the presence of BGY fluorescence followed by statistical analyses of the differences in expression of fluorescence between the various isolates. Preliminary results indicate that all aflatoxigenic fungi may not necessarily induce the expression of BGY fluorescence in corn kernels even after extended, post-inoculation incubation period. Results are expected to deepen our understanding concerning the association of BGYF with the presence of aflatoxin in different aflatoxigenic fungal strains.

P58: Approach to evaluate the impact of different structural moieties of ochratoxin A on its cytotoxicity by testing synthesized derivatives

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Ochratoxin A (OTA) has a widespread occurrence in food. It is known that OTA is nephrotoxic and it is classified as a group 2B carcinogen. However, the mechanism why the mycotoxin impairs cell and organ function is still not solved in detail. All indicates that an interaction with target molecules is necessary for any observed adverse effect. This interaction depends on characteristics of the target molecule as well as on the OTA molecule itself. OTA has different structural moieties which may be relevant for these interrelations including a halogen (chlorine) and an amino acid group (phenylalanine). To test their importance for the impact of OTA detailed structure-activity studies were performed. For this 23 OTA derivatives were available, which were modified by either an exchange of the halogen moiety against another halogen (fluorine, iodine or bromine) or by the amino acid moiety against another one (tyrosine or alanine) or a combination of both. Additionally, the configuration of the 3R carbon atom was changed to 3S. These derivatives were tested in human renal cells for their ability to induce cell death (cytotoxicity, apoptosis, necrosis). These experiments led to the result that the substitution of the amino acid moiety against tyrosine or alanine almost completely prevented the adverse effects of OTA. The exchange of the halogen moiety had minor effects and the inversion of the stereochemistry at C3 did not prevent the OTA effects. Therefore, we conclude that the amino acid moiety of OTA is a prerequisite for the interaction of OTA with its target molecules. Acknowledgements. Supported by the Deutsche Forschungsgemeinschaft (DFG) HU 730/12-1 and SCHW 1515/2-1).
P59: Bioactive compounds from fungal cultures: identification, isolation and structure elucidation

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Fungi are major plant and insect pathogens since they produce a multitude of low-molecular-mass compounds known as mycotoxins. Among others Penicillium and Fusarium genera are important toxigenic fungi, which are relevant in food products (Sweeney and Dobson, 1998). Nevertheless, some species of these genera are not well characterized and can produce several unknown metabolites, thus making them a promising source for the discovery of new bioactive compounds. Our approach ranges from the development of cultivation conditions to genetic engineering, including overexpression and deletion mutants of biosynthetic genes and/or global regulator genes followed by identification via HPLC-UV-HRMS and isolation. To enhance this identification process we used software-assisted tools (MZmine 2) to analyze the respective metabolomes (Pluskal et al., 2010). Isolation and separation steps were carried out with techniques such as flash chromatography, preparative TLC and HPLC, solid phase (SPE) and liquid-liquid extractions. The structures of isolated compounds were elucidated by NMR, IR, CD-spectroscopy and fragmentation experiments using HPLC-HRMS. New identified mycotoxins were screened for their cytotoxicity against different human primary as well as cancer cell lines using the Cell Counting Kit-8 (CCK-8) assay. For the further understanding of molecular mechanisms underlying the metabolites bioactivity and toxicity, computational methods for target prediction and molecular docking studies were performed. The results for several known and unknown secondary metabolites isolated from Fusarium and Penicillium species will be presented.

P60: Design of a novel microfluidics platform for mycotoxin food contaminant determination

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Many current methods for mycotoxin analysis are costly and require skilled personnel using laborious analytical methods in laboratory facilities. There is a pressing need for the development of a portable, cost-effective, highly sensitive multiplex method of mycotoxin analysis to protect consumers. Achieving this will encompass production, isolation and engineering of a panel of specific anti-mycotoxin recombinant antibody fragments. This project will utilize these antibodies to design a test capable of detecting a panel of key mycotoxins including aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), patulin, deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin. Currently, a highly sensitive Fab antibody has been engineered to target AFB1 with a LOD below legislative limits. At present, antibody libraries from an avian host are being screened to isolate antibody fragments with high affinity to AFM1 and patulin. Furthermore, there is a lack of detailed understanding of how mycotoxins, alone and in combinations, interact with the immune system. This project aims to address this issue by examining the effects of mycotoxin exposure on some of the key cells involved. Thus far, this project has examined the effect of patulin, DON, ZEN and T-2 toxin exposure on the secretion of key cytokines from the murine macrophage cell line, J774A.1. Exposure of macrophages to these mycotoxins resulted in a dose-dependent modulation of cytokine secretion. Specifically, exposure to low doses of patulin (0.001, 0.1 and 1 pg/ml) resulted in a statistically significant decrease in the secretion of the pro-inflammatory cytokines interleukin (IL) 6 (IL-6) and tumor necrosis factor alpha (TNF-α), following stimulation with lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls. Treatment with low doses of DON (0.001 pg/ml) and ZEN (0.001 and 0.01 pg/ml) significantly decreased (p<0.01) the secretion of the pro-inflammatory cytokine IL-12p40, while several doses of T-2 toxin (0.001, 0.01, 0.1, 1 and 100 pg/ml) caused a significant decrease in the expression of IL-6. Each of the mycotoxins also significantly increased the production of the anti-inflammatory cytokine IL-10, both before and after LPS stimulation. This data provides further insight into the mechanisms by which mycotoxins modulate the host immune response. Further development of this work followed by successful incorporation of such measurements, in combination with total mycotoxin determination, on an easy-to-use detection device will be a significant advancement in mycotoxin analysis. The project will design a test capable of quantifying multiple mycotoxins in addition to measuring their potential synergistic effects in food samples. This strategy will move mycotoxin analysis from the lab to the field allowing for early intervention to improve food safety globally.
P61: High throughput automated cleanup and analysis of aflatoxin and ochratoxin

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Regulations for mycotoxins are complex, with different toxins associated with specific commodities. For matrices like dried fruit and spices, there are legislative limits in place for aflatoxins and ochratoxin meaning that analysts are required to routinely analyze such samples for both mycotoxins. In order to maximize sample output, a simple generic extraction for both aflatoxin and ochratoxin was performed using QuEChERS salts. A wide range of cereal, nuts, dried fruit and spice samples spiked at levels in line with EU legislation were extracted, before being cleaned up and analyzed automatically using the new IMMUNOPREP® ONLINE aflatoxin or ochratoxin cartridges. IMMUNOPREP® ONLINE cartridges contain a monoclonal antibody for the mycotoxin of interest coupled to a rigid hydrophilic sorbent, which means that each cartridge is not only highly specific but can also withstand high pressure allowing the cartridge to be switched in line with an HPLC column. Sample application, washing and elution can be performed automatically with the RIDA®CREST handling system. Each IMMUNOPREP® ONLINE cartridge is calibrated and can be used for up to 12 samples before the cartridge is automatically removed and replaced with a new one. Using IMMUNOPREP® ONLINE, excellent chromatography was achieved and repeatability, reproducibility and recoveries were found to exceed EU performance criteria for both mycotoxins in the above matrices.

P62: Incorporation of recombinant Fab antibody fragments in a ‘point-of-site’, optical-planar waveguide biosensor device for detection of aflatoxin B1

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Mycotoxins are naturally occurring food adulterants produced by Aspergillus and Fusarium species. These toxins have huge implications for food safety readily contaminating at least 25% of globally consumed basic food commodities such as rice, corn and wheat (Boutrif and Canet, 1998). When ingested, mycotoxins may cause mycotoxicosis which can provoke acute or chronic disease. Chronic exposure has a greater impact on health stimulating carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, dermotoxic and neurotoxic effects in humans and animals. A report in 2013 from The United Nations indicated that global population is projected to increase by almost one billion people reaching 8.1 billion by 2025, and to further increase to 9.6 billion by 2050 (United Nations, 2013). Due to the severe mycotoxic consequences on health and the increasing focus on food-safety to support the global population, prodigious efforts are being made to control and eliminate the risks of mycotoxin-contamination. Consequently, mycotoxin analysis is now considered one of the most important areas for food quality improvement. There is currently an urgent requirement for highly-sensitive, rapid, in-expensive point-of-site detection methodologies for these toxins to control the risk of contamination in foods. This study describes the bacterial expression and purification of an anti-aflatoxin B1 recombinant antibody Fab fragment for incorporation in an optical-planar waveguide biosensor device for sensitively detection. The anti-aflatoxin B1 recombinant Fab antibody fragment can achieve an IC50 value of 2.9 μg/kg and a limit of detection of 1.9 μg/kg in competitive inhibition ELISA. This limit of detection is lower than the current maximal levels of 2-4 μg/kg outlined by the European Union for aflatoxin B1 in cereals intended for direct human consumption (Commission Regulation (EC) No 1881/2006) and 20 μg/kg outlined by the U.S. Food and Drug Administration (USFDA) for total aflatoxins (B1, B2, G1 and G2) products designated for humans except milk (USFDA, 2005). Therefore, through the incorporation of the anti-aflatoxin B1 Fab within a ‘point-of-site’, optical-planar waveguide biosensor device the detection of AFB1 in foods to EU and USFDA standards is anticipated. This system will take sensitive mycotoxin analysis out of the traditional laboratory-based setting and directly into the field permitting monitoring and identification of unsafe food for human and animal consumption.
P63: Development of method for the simultaneous determination of multi-mycotoxins in green coffee bean by LC-MS/MS after SPE cleanup

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A new method for the simultaneous detection of multi-mycotoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1 aflatoxin G2, ochratoxinA, fumonisin B1, fumonisin B2 and zearalenone) in biscuit, infant food, peanut and raisin was internally validated. This method is based on SPE purification after single extraction and detection using liquid chromatography tandem mass spectrometry (LC-MS/MS). Matrix-matched standard calibrations were used for quantification. The ratio (slope of matrix-matched calibration, B/slope of the standard calibration in solvent, A×100) was defined as the matrix effect (ME). The matrix effect (ME) was calculated for each mycotoxin in biscuit, infant food, peanut and raisin. The correlation coefficients (r²) were higher than 0.996. The results of the recovery test showed in the range 84.5-123.5% with relative standard deviations ranged between 2.0-32.7% (RSDR). Limits of detection and quantification were also estimated 0.25-10 ng/g and 0.5-20 ng/g, respectively. The developed method was performed first for biscuit, infant food, peanut and raisin, and found suitable to determine the multi-mycotoxins with satisfactory intensities and accuracy.

P64: Fit-for-purpose immunochemical test kits for fumonisins screening – different solutions for different needs

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The aim of the present work is to present the performances of three different immunoassays developed for the screening of fumonisins in corn and other matrices. Celer® FUMO is a quantitative ELISA test kit designed for the analysis of feed and corn by-products intended for animal consumption, meeting the EU and FDA fumonisins limits in these matrices ranging between 1000 and 60,000 μg/kg. Celer® FUMO measuring range is 750-60,000 μg/kg indeed. Since it is addressed to farms, breeders and industries, the assay is fast: it takes 20 min. The assay was validated according to Commission Regulation (EU) No 519/2014 by setting the screening target concentration at 1000 μg/kg. 20 blank corn samples (<250 μg/kg, HPLC analysis) were analyzed to set the specificity of the assay in matrix. 100% specificity was obtained, since no false positive results were obtained. The same samples were spiked with 1000 μg/kg of fumonisin B1: all determinations were revealed as positive by the assay, with no false compliant results. The bias was evaluated by analyzing 150-47,000 μg/kg contaminated incurred samples, the recovery was 102±23% (n=60). I’screen FUMO is a supersensitive quantitative ELISA test kit conceived for the analysis of corn for human consumption, where EU and FDA limits range between 200 and 4,000 μg/kg. The assay measuring range is far more sensitive: 25-1000 μg/kg, extendable to 5,000 μg/kg by dilution. Due to its high sensitivity, it was not possible to set any screening target concentration and to analyze the matrix effect of blank corn samples, since no material with concentration lower than 150 μg/kg was found. The accuracy of the assay was hence evaluated by analyzing 250-5,000 μg/kg contaminated incurred samples. The mean recovery was 115±24% (n=45). Smart Strip FUMO is a quantitative lateral flow device designed for easy and fast corn acceptance control. The main requirement for the development of this assay was quickness, therefore the analysis takes 5 min only. The test measuring range is 150-4,000 μg/kg, to be extended to 20,000 μg/kg by dilution: such measuring range can fit the need for analysis of raw materials for food and feed production. For the same reason mentioned above, no blank samples were available for the investigation of matrix effect and the sensitivity of the assay. The performances of the kit were investigated by analyzing 150-5,000 μg/kg contaminated incurred samples. The mean recovery was 110±29% (n=78).

P65: On-site study to evaluate rapid inspection of grain trucks on mycotoxins based on dust sampling

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Mycotoxins are ubiquitous in grain and can never be completely avoided. Especially in years with increased incidence of mycotoxins, a reliable control at grain intake is essential to ensure that purchased grain meets legal requirements and thus is marketable. However, particularly in season, trucks deliver at high frequency limiting the duration for intake control to at most 10-15 min. Rapid tests on mycotoxins that take only 5 min for analysis are available. However, the time to prepare a proper sample is the
limiting factor. For sufficient control 5-10 kg of grain should be sampled per truck from at least 5-8 different points. Only grinding of the whole sample to <1 mm assures representative tests yielding reliable results. However, grinding 5-10 kg of wheat takes up to one, the same portion of corn even up to three hours. Hence, in current praxis either every truck is directly controlled, but based on a far too small sample or lots are tested based on a representative amount, but results are received when the grain is already in the silo. Both compromises bear high risk to accept contaminated lots that afterwards need to be separated at high effort and costs. A solution is dust sampling. Sampled grains (5-10 kg) are directly poured into a sieve tower. After 2-3 min a representative dust sample is obtained. This dust results from abrasion of all kernels in the sample. Grain dust is analyzed by standard lateral-flow devices, but the obtained result has to be recalculated from the mycotoxin content in the dust to the concentration in the grain. This approach was tested on-site under real conditions. At grain-elevator stations wheat was analyzed on deoxynivalenol and corn additionally on aflatoxins using the rapidust system®. Tests were carried out by persons working at the elevator. Some even had no laboratory experience. The decision on acceptance or rejection of a lot was supported by an application signaling mycotoxin levels in a traffic light system. Afterwards, the results for kernels identified as contaminated were double checked by HPLC-MS/MS in a laboratory. Even the untrained staff were able to carry out mycotoxin control within 10-15 min and take immediate decisions on acceptance or rejection. Up to 18 trucks were controlled continuously at this frequency. Results obtained by the rapidust system® were similar to results obtained after analyses of the respective kernel samples in the lab.

P66: Rapid dust screening by means of LC-MS/MS to obtain occurrence patterns of Fusarium, Aspergillus, Penicillium and Alternaria toxins in corn

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Multiple factors as are rain during flowering, insect damage, or storage at high humidity favor the pre- and postharvest infection of corn with molds. Main mycotoxin producing genera are Fusarium, Aspergillus, Penicillium and Alternaria. According to the prevalent strain and environmental conditions these molds produce a wide range of toxins. However, which mycotoxins are produced to what extent can hardly be predicted. Multi-mycotoxin screening methods are best suited to reveal contamination pattern of corn lots. Results are needed at the beginning of each harvest season to set up sufficient risk-based sampling plans and identify the right toxins to focus on in daily control. Furthermore, occurrence pattern can help to obtain data on emerging toxins and deduce the risk for a specific cropping region. Thereby, methods have to be simple, fast and cost-efficient. Mycotoxins accumulate on small particles in food or feed bulk. Consequently, dusts are often higher contaminated with mycotoxins than the grain kernels. By using the advantages of dust sampling, a dilute and shoot LC-MS/MS approach was set up to screen corn samples for more than 30 mycotoxins including A- and B-type trichothecenes, zearalenone, fumonisins, enniatins, ochratoxin A, aflatoxins, and Alternaria toxins. More than 100 samples from Germany, Poland, Romania, France and further European countries harvested in 2011-2015 were analyzed. All mycotoxin concentrations were plotted in color intensity charts to reveal occurrence patterns. While middle European samples showed mainly Fusarium toxins, a prevalence of Aspergillus toxins was observed in southern European corn in 2013 harvest. Fusarium toxins always comprised deoxynivalenol, often accompanied by its 15-acetyl and sometimes also 3-acetyl derivative as well as nivalenol. Many samples contained also zearalenone and fumonisins. Besides, enniatins were detected in every sample with beauvericin showing highest concentrations. In contrast, fusarenon-X was hardly ever observed. Aflatoxins often co-occurred with ochratoxin A but also sterigmatocystin, cyclopiazonic and mycophenolic acid. The latter was also found together with roquefortine C in absence of aflatoxins in wet corn intended for silage. Alternaria toxins were observed in many samples as well, in which tenuazonic acid dominated followed by alternariol and alternariol monomethyl ether. AAL-toxin was not significantly detected in corn samples. In conclusion, dust sampling combined with LC-MS/MS multi-toxin screening was shown to give a comprehensive overview on mycotoxin co-occurrence patterns in corn. Hence, this technology has a huge impact on cost management for risk assessment since sampling strategy and toxin analysis can be focused on identified toxins.
P67: Co-occurrence of *Fusarium, Aspergillus, Penicillium, Alternaria* toxins and ergot alkaloids in European wheat and rye samples screened by means of dust analyses

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Often grain is naturally contaminated with several mycotoxins. Hence, the co-occurrence of toxins has become of main toxicological interest. Also from an analytical point of view occurrence patterns helps to identify potential indicator-toxins or design risk-based test plans. Many mycotoxins are predominantly produced in the outer kernel layers. Hence, abraded particles from the surface are often higher contaminated with mycotoxins compared to the grain kernels. Other toxins as ergot alkaloids accumulate on small particles in the bulk due to their higher surface to volume ratio. The natural enhancement of toxins in the dust can facilitate analysis and enable simple dilute and shoot LC-MS/MS screening methods. By means of dust sampling combined with LC-MS/MS multi-toxin screening a comprehensive overview of co-occurrence of mycotoxins in wheat and rye samples could be given. More than 100 wheat and ~40 rye samples from Germany, Poland, France and further European countries harvested in 2011-2015 were analyzed. Occurrence patterns of 40 toxins were shown in color intensity charts based on concentrations in the grain dust. *Fusarium* toxins were found in every sample with deoxynivalenol dominating. In addition to the latter, the samples often contained also 3-acetyl and/or 15-acetyl-deoxynivalenol and/or nivalenol. However, amount and occurrence was hardly correlated with the former. Also, zearalenone occurrence could not be deduced from deoxynivalenol, although no sample exclusively containing zearalenone was found. Whereas T-2 and HT-2 toxin were observed in many samples, fusarenone-X as well as mono- and diacetoxyisocerpenol were hardly detected. In contrast, enniatins were found to be ubiquitous. Of the latter, enniatin B prevailed. Aflatoxins do not play a role in European wheat and rye samples. In contrast, ochratoxin A analyses are always crucial. Traces of *Alternaria* toxins were found in many wheat samples. Some contained tenuazonic acid, alternariol, alternariol monomethyl ester, altemuene and tentoxin, other only some of the latter in various combinations. AAL-toxin was not detected in any sample. Also within the ergot alkaloids no marker toxin could be identified. In fact, ergot alkaloid patterns differed from sample to sample showing the need for specific methods including all derivatives. Summing up, detailed multi-toxin analyses remain the only possibility to assess the contamination of a grain sample correctly. Thereby, the presented quick and cost-efficient pre-screening using the dust sample can help to focus on the right toxins. Toxins not detected in the enriched dust fraction are extremely unlikely to be present in the grain.

P68: Detection of ochratoxin A contamination in stored wheat and barley using near-infrared (NIR) hyperspectral imaging system

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An NIR hyperspectral imaging system was used to detect different concentration levels of ochratoxin A contamination in wheat and barley. The wheat and barley grains were artificially infected with Ochratoxin A producing *Penicillium verrucosum* strain and the infected kernels were tested for presence of ochratoxin A 18, 20, 22, 24, and 26 weeks post-inoculation. The wheat and barley kernels with known amount of ochratoxin A concentrations and sterile control samples were subjected to single kernel imaging. The acquired image data were preprocessed and transformed into readable two-dimensional form. Principal Component Analysis was applied to the two-dimensional data to identify significant wavelengths. Statistical and histogram features corresponding to significant wavelengths were extracted and used in statistical discriminant pair-wise, two-class, and six-class models. The statistical classifiers differentiated sterile control samples from different concentrations of ochratoxin A contaminated kernels with a classification accuracy of 100%. Image data from non-ochratoxin A producing *P. verrucosum* strain infected wheat and barley kernels were compared with ochratoxin A contaminated wheat and barley kernels with a classification accuracy of 99%.
P69: Analysis of patulin in apple-based products using molecularly imprinted polymer sample preparation and fast UHPLC detection method

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Patulin is a mycotoxin produced by a number of fungal species in rotten fruits, specifically, apples. A sensitive and fast method for low level patulin detection in apple juice and apple puree is demonstrated herein. The sample preparation methodology utilized molecularly imprinted polymer (MIP) cleanup allowing for easy and effective separation of patulin from matrix interferences. MIP solid phase extraction material is engineered to selectively bind the analyte of interest while strong washes can be applied to remove other matrix components resulting in cleaner samples. Analysis of patulin was done by UV detection in less than 7 min using HPLC column packed with sub-2 micron monodisperse porous particles. The method was demonstrated for apple juice samples spiked at 50 ng/ml and 10 ng/ml and for apple puree samples spiked at 10 ng/ml. Excellent patulin recovery and reproducibility was observed from the various matrices and will be described.

P70: Effect of sample matrix on ionization effects during LC-MS analysis of mycotoxins in corn and animal feed

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The use of mass spectrometry methods for multi-mycotoxin analyses provides high levels of detection selectivity and sensitivity. In this work two samples, extracts of corn and of goat feed, were spiked with 15 different mycotoxins to investigate the effects that these two matrices impose on the accuracy of quantitation. The samples underwent a fast extraction procedure and dilution prior to injection into LC-MS/MS instrument. As no sample cleanup was applied the separation of analytes from matrix became important to reduce the possible matrix ionization effects. The UHPLC separation of mycotoxins was performed using phenyl-hexyl HPLC column chemistry. Most compounds were found to be stronger retained and better separated from the matrix in comparison to the more commonly used C18 columns. The results indicated that for most studied mycotoxins, the co-extracted matrix did not contribute to significant ionization effects when the phenyl-hexyl stationary HPLC phase was used. The matrix ionization effects were higher for aflatoxins and DON in the goat feed matrix in comparison to these in corn due to higher matrix complexity of the former sample.

P71: Analysis of mycotoxins in cereals using a simple extraction and LC-ESI/MS/MS with fast polarity switching and scheduled MRMs (multiple reaction monitoring)

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A quick and sensitive LC-MS/MS method was developed for the detection of several major classes of known toxic and regulated mycotoxins. The method uses a simple solvent extraction followed by a further dilution and injection of sample extracts to achieve detection of mycotoxins below regulatory requirements (sub-ppb levels). A Waters I-Class UPLC with a Phenomenex Kinetex 2.6µm XB-C18 column, integrated with an AB Sciex Triple Quad 5500 using fast polarity switching and Scheduled MRMs allows detection and quantitation for all the mycotoxins of interest. Of the 25 mycotoxins analyzed, 17 compounds were detected in the positive ionization mode and 8 in the negative ionization mode. Quantitation employs both C13 internal and matrix matched standards. Two precursor product ion transitions are used to quantify and confirm results. This method has been validated for cereal crops (wheat, barley, oats and corn). Over the past three years, the method has demonstrated acceptable results for numerous proficiency testing programs (FAPAS, MoniQA, CODA-CERVA and Neogen).
**P72: Optimized LC-MS method for multi-mycotoxin analysis in human plasma for exposure studies of Canadian population**

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Filamentous fungi produce toxic compounds known as mycotoxins. The contamination of food commodities with low levels of mycotoxins is widespread in both food and feed, including wine, cereals, milk, coffee beans, corn, nuts and dried fruits. Numerous studies show that the deleterious effects of mycotoxins on human and animal health represent an important issue for further study. Since, there is no surveillance data on Canadian population for most mycotoxins using direct monitoring of biological fluids, it is important to assess human exposure to common mycotoxins to help assess effectiveness of current regulatory measures. The objective of this work was to develop a sensitive and reliable multi-mycotoxin assay allowing simultaneous detection and quantification of common toxicologically important mycotoxins and their metabolites in human plasma. The developed method used liquid chromatography-mass spectrometry (LC-MS) in combination with a universal sample preparation technique in order to achieve high sensitivity, speed, and accuracy. Blood was selected as the matrix for this analysis as it provides better readout of long-term exposure to mycotoxins, at least for mycotoxins that have long plasma half-life. Excellent sensitivity is necessary for the method due to the fact that mycotoxins in plasma are present in pg/ml concentrations. The optimization of LC-MS method included a detailed comparison of sample preparation techniques, sample pH, chromatography, and LC-MS instrumentation. In order to obtain high analyte recovery, good selectivity and to minimize matrix effects several types of sample preparation techniques were investigated: solvent precipitation with acetonitrile, solid-phase extraction (strong anion exchange, hydrophilic-lipophilic-balanced (Oasis HLB) sorbent and mixed mode sorbent (Bond Elute Certify II)), and liquid-liquid extraction (ethyl acetate versus methyl tert-butyl ether). The effective separation of mycotoxins including isomers was achieved with pentafluorophenyl column and the mobile phase containing water/methanol with 0.1% acetic acid and water/methanol with 0.02% acetic acid for positive and negative electrospray ionization (ESI), respectively. The optimized method was validated according to regulatory guidelines and is suitable for mycotoxin exposure studies.

**P73: Development of nanosensor platforms for mycotoxins detection**

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Mycotoxins contamination in foods is of global concern owing to their potential toxicity, carcinogenicity and immunosuppressive effect on mammalian systems. A major goal for consumers, food producers and agro-food companies is to prevent and control mycotoxins in foods and feeds for human and animal health safety. Hence, concern has been expressed for the detrimental effect aflatoxin B1 (AFB1) can have, since this toxin is well known to be the most predominant and toxic fungal toxin. Therefore, AFB1 analysis methods must be able to detect the compound with high sensitivity, selectivity and accuracy. This also highlights the need for the development of on-site methods that are portable, rapid and exhibit the specificity and low detection capability of conventional methods. This poster reports on the development of a nanosensor for AFB1 detection that combines a highly sensitive gold chip sensor with gold nanoparticles signal amplification. The sensor with the optimized assay showed high sensitivity with detection limit (LOD) of 1ng/l for AFB1. Molecular imprinted nanoparticles (nanoMIPs) are also being developed to replace the antibody used as the detection molecule for the development of a more stable nanosensor for field analysis.

**P74: Performance of grinders and dividers for preparing whole oats for mycotoxin analysis**

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The grinding and division of oats can be a difficult and time consuming procedure that can affect the particle size and homogeneity. This ultimately affects the analysis of mycotoxins. The Canadian Grain Commission provides a unique setting in which all of these factors can be investigated. The purpose of this study was to evaluate particle size distribution for four grinders and four dividing methods to determine which grinder setting and dividing method would be most effective. This will provide
information to guide advice on grinders that produce the 'best' (i.e., smallest) particle sizes for groups doing mycotoxin analysis. Oat samples were ground using four different grinders; the Ditting, Retsch, Romer and Perten grinders. The Ditting and Romer were assessed at different grind settings, from the finest setting to the coarsest, while the Retsch and Perten were using different screens (500, 750 and 1000 μm, and 500 and 750 μm, respectively). Grinders were evaluated for the following parameters; time required to grind 1 kg, time to clean between samples, amount of sample loss, and various ‘ease of use’ issues (clogging, overheating, etc.). Based on grinder assessment samples from the most and least efficient grinder were subjected to several dividers; Boerner, Riffle, Rotary sample divider (RSD) and a manual dividing method. Dividing methods were evaluated for their time to divide 1 kg samples, the time to clean between each sample and various ‘ease of use’ issues. The particle size distribution of the divided samples was measured to verify particle size consistency. Particle size distribution was measured by mechanically sifting the sample through a box sifter comprised of a series of stacked mesh screens of various sized openings (1180 μm, 475 μm, 300 μm, 180 μm and 110 μm). After set periods of time the material remaining on the top of each screen was collected and weighed. The results from this study showed that that the Perten with a 500 μm screen gave the best (smallest) particle size; however, problems with over-heating and constant cleaning were required with samples greater than 200 g. The Retsch with a 500 μm screen provided the second best in terms of particle size but was far more practical based on the aforementioned criteria. Additionally, the Riffle divider was the most efficient in terms of ease of use and speed. Finally, there was no notable difference when evaluating particle size consistency between each dividing method.

P75: Application of biomolecular methods for the quantification of mildew damage and its causal agents in red spring wheat from Western Canada

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Mildew damage in Canada Western Red Spring (CWRS) wheat is currently assessed on a visual and subjective inspection by trained inspectors from the Canadian Grain Commission (CGC). Mildew damage is an important degrading factor because of its detrimental effects on the quality of grain end products such as flours, breads and pastas. Higher levels of mildew equate to higher discoloration in end products and can be associated with Alternaria mycotoxins such as alternariol. This can also be detrimental to a producer’s compensation. The present study investigated the relationships between grade of mildew damaged Canada Western Red Spring (CWRS) wheat and quantities of Alternaria biomass as well as mycotoxins. Composite samples were assembled to represent a combination of all four grades (1, 2, 3, and feed), locale (province and crop district) and variety. Samples were quantified through bio-molecular methods employing real time polymerase chain reaction (qPCR) to determine the correlation between grade and fungal load associated with mildew. Composite samples were analyzed and quantified for the following factors: Alternaria alternata, A. infectoria, Cladosporium species and alternariol (a mycotoxin produced mainly by Alternaria species). Mycotoxin levels were compared to results of the fungal qPCR analyses. Results from this study showed that A. alternata was consistently the most commonly found species associated with mildew damaged wheat. In western parts of the Canadian Prairies, A. triticina and other closely related species were also frequently detected. Cladosporium species were detected only at low levels across Western Canada. In addition, samples from Manitoba displayed the highest levels of A. alternata with a positive correlation between fungal load and decreasing grade.

P76: Development of an immunoaffinity column for sterigmatocystin analysis using an organic solvent-tolerant monoclonal antibody

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Sterigmatocystin (STC) is a mycotoxin produced by several Aspergillus species, an intermediate in the aflatoxin metabolic pathway. It is commonly known as a contaminant of grain products. A monoclonal antibody specific to STC was prepared. In an indirect competitive enzyme-linked immunoabsorbent assay, the half maximal inhibitory concentration (IC50) values were 0.45, 0.45, 0.72, 1.02, and 5.26 ng/ml for aflatoxin (AF) B1, AFB2, AFG1, AFG2, and STC. This antibody showed reactivity with aflatoxins. Next, an immunoaffinity column (IAC) was prepared using the antibody by binding it with agarose gel beads. The recovery of STC from this IAC was over 80% when STC was extracted in a solvent of up to 20% methanol concentration or up to 10% acetonitrile concentration. The results show that the IAC with this
antibody has high organic solvent tolerance. The IAC using the organic solvent-tolerant monoclonal antibody can be used to much wider range of samples.

P77: Development and application of an accurate mass LC-MS/HRMS library for the screening of mycotoxins and other fungal metabolites in food and feed

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Methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of mycotoxins in different commodities are steadily increasing in popularity. With regard to the high number of possible target analytes and the still limited availability of commercial standards, alternatives for these methods are necessary. LC coupled to a high resolution mass spectrometer (HRMS) allows the screening of a, theoretically, unlimited number of contaminants along with the retrospective search for novel compounds of interest. A database for mycotoxins and other fungal metabolites containing more than 400 entries was created. For 270 compounds MS/HRMS-spectra were successfully acquired in positive and/or negative electrospray ionization mode at three different collision energies with an Agilent 6550 iFunnel QTOF. The obtained accurate mass spectra were corrected to their theoretical mass-to-charge ratio by a probabilistic approach before incorporation into the library. Sample preparation was based on a 5 min extraction with acidified acetonitrile-water, without any sample cleanup. The analyte separation was achieved using a water-methanol gradient on a reversed phase column with a total run time of 15 min. Three different screening approaches were applied and the following respective methods developed: In the classical screening approach, an HRMS scan is performed first, followed by a database search and a targeted MS/HRMS acquisition of the suspected compounds with a second chromatographic run. The acquired MS/HRMS spectra are then compared with the library spectra to eliminate false positive suspects. The second possibility is to define a target inclusion list and if certain criteria are fulfilled, MS/HRMS spectra are automatically triggered (directed MS/HRMS mode). This approach requires only one chromatographic run per polarity. The third approach is called ’All Ions’ and allows the acquisition of HRMS and MS/HRMS spectra within a single injection by fragmentation without precursor selection. In ’All Ions’ mode the presence of mycotoxins is confirmed by the co-elution of characteristic fragment ions deposited in the library. To cross-validate the approaches, three different matrices were chosen and extracts were spiked with different levels of a subset of the fungal metabolites included in the library. Concluding, the LC-QTOF method in combination with the generated LC-MS/HRMS database and library is a suitable tool for the screening of mycotoxins and other fungal metabolites in food and feed. Moreover, by utilizing this approach the need for authentic standards to confirm the identity of the measured analytes becomes increasingly irrelevant if identical instrumental setup is used in different laboratories.

P78: Afla-V AQUA quantitative lateral strip test for aflatoxin in corn

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Aflatoxins are naturally occurring chemical byproducts of mold species, which are natural inhabitants of crop soils. Severe rain and drought can encourage aflatoxin production. Aflatoxin contamination of grains and agro-products is a serious food safety issue and a significant economic concern worldwide. The USDA, FDA, EU, and other international government agencies impose specific regulations and guidelines for acceptable levels of aflatoxins. Producers and exporters use on-site and laboratory testing to ensure regulatory compliance and to protect human and animal health from illnesses associated with aflatoxins. To rapidly screen for aflatoxins in grain products such as corn, VICAM has developed the Afla-V AQUA quantitative lateral strip test utilizing the company’s proprietary monoclonal antibodies. Afla-V AQUA eliminates any organic solvent (ethanol or methanol) by using an aqueous, eco-friendly solution for aflatoxin extraction. Overall test time from sample extraction to concentration reading is less than 10 min. Afla-V AQUA was developed and validated in accordance with the Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits as specified by the USDA-GIPSA. Its sensitivity (limit of detection, LOD) was determined as low as 2 μg/kg and its working range was from 2 μg/kg to 100 μg/kg. Analysis of naturally contaminated samples matched well with HPLC analysis (r²=0.99). Inter-operator reproducibility was studied by three operators using four naturally contaminated corn samples analyzed by HPLC, and its temperature sensitivity was also evaluated at 18°C, 24°C and 30°C, all data were within GIPSA specifications.
P79: Single/multi-laboratory evaluations of mycotoxin analysis in foods by liquid chromatography-mass spectrometry

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To ensure food supplies are safe the U.S. Food and Drug Administration (FDA) laboratories have been routinely monitoring regulated mycotoxins in a variety of foods and feeds. The current analytical methods used are based on LC-UV/fluorescence technologies and only suited for the analysis of single or single-class of mycotoxins. For suspected violations, an additional confirmation procedure using mass spectrometry is required. Furthermore, as co-contamination with different mycotoxins in foods and feeds may pose a serious threat to humans and animals, the agency needs to simultaneously monitor different mycotoxins in food products in a more time-efficient manner and thus prevent adulterated products from reaching consumers. In recent years, we have been conducting a series of single- and multi-laboratory method development and validation studies to evaluate the applicability of LC-MS based methods. For single-laboratory validation, we have assessed the performance of LC-MS/MS and LC-HRMS and sample preparation procedures (e.g., immunoaffinity clean-up, dilute-and-shoot) in cereals, nuts, dried fruits, rice, wheat flour, milk, juices, and baby foods. For multi-laboratory studies, the U.S. FDA and state laboratories in the U.S. have developed and validated a stable isotope dilution LC-MS/MS method that targets 12 mycotoxins (e.g., aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, HT-2 and T-2 toxin and zearalenone) of regulatory and health significance in corn, peanut butter, and wheat flour. These newly validated methods can identify and quantitate mycotoxins in different matrices using a single sample preparation procedure and LC-MS analysis. If used for routine regulatory monitoring and surveillance, these methods would significantly improve the mycotoxin screening efficiency of the FDA laboratories in terms of identification, quantitation and sample throughput. This would enable the agency to efficiently identify foods and feeds that may warrant regulatory actions. It is worth noting that there are still challenging issues related to LC-MS based multi-mycotoxin analysis methods such as availability, stability, and traceability of the standards, automated sample preparation, matrix effects, identification criteria and harmonization of existing analytical procedures. This presentation will discuss these important but underexplored issues that we have encountered in the course of method development and validation and propose practical solutions for the future studies.

PREVENTION AND CONTROL
P80 – P94

P80: Biocontrol of Aspergillus flavus: the Italian experience

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Aflatoxin contamination in corn is one of the hottest agricultural topics in Italy. Carry-over of aflatoxins from animal feed to milk and milk products causes severe economic loss. Aflatoxins put pressure on corn, milk, and cheese producers and there are no simple and effective preventative procedures for the industry to follow. Indeed, traditional management procedures, such as application of fungicides and genetic resistance, are ineffective. Biocontrol, implemented with select non-aflatoxigenic genotypes of Aspergillus flavus, has been amply demonstrated as effective in the U.S. and in Africa where commercial biopesticide products are available. The candidate genotype for biocontrol must (1) be native to the area in which it will be used, (2) have a genetic defect that eliminates its ability to produce the target mycotoxins, and (3) be very competitive against toxigenic genotypes. Severe aflatoxin contamination in Italian corn was detected for the first time in 2003 and since that year the selection of genotypes for potential use as biocontrol agents has been underway. Non-aflatoxigenic individuals were selected from about 150 isolates of A. flavus and confirmed with DNA methods. Two candidate genotypes lacking all the biosynthesis genes required for production of aflatoxins and cyclopiazonic acid were selected for further work after evaluation of genotype competitiveness and ability to reduce aflatoxin biosynthesis in vitro. In 2012, field trials were performed utilizing these two genotypes as active ingredients in order to determine extents of efficacy on corn during the natural contamination process. When compared to untreated controls, the treatments caused a statistically significant reduction in aflatoxins of approximately 90%. However, one of the two non-aflatoxigenic A. flavus genotypes was recovered from the grain at a much greater frequency than the other. Based on these results, only a single genotype was selected for further study and development as a biopesticide Italian corn. This A. flavus genotype
was deposited in May 2013 at the MUCL fungal collection (MUCL54911) and a patent for the biopesticide was issued in September 2015, N.001417885. Although the initial research in developing this biocontrol for Italy is now complete, there will still be a long path until there is a tool Italian farmers can use. A portion of this path involves registration of the biopesticide which contains the selected A. flavus genotype as an active ingredient. A temporary authorization for use of this biopesticide in commercial agriculture was approved last year and this approval will allow broader field evaluations during 2016.

P81: High moisture corn ensiling is a process conducive to fumonisin B1 biodegradation

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Fusarium verticillioides and F. proliferatum are two major epiphytic fungi of corn that can produce mycotoxins, such as fumonisin B1 (FB1). Corn silage that is based on the fermentation of whole crop plant or grains represents one of the primary feed ingredients of monogastric and ruminant livestock. The ensiling process could favor changes in FB1 content. Both increases or decreases of FB1 have been reported following ensiling process. However, this has scarcely been documented. The possibility of managing the microbiota while ensiling to reduce the level of FB1 exposure and improve feed quality could be a promising option. The present work aims at studying the fate of FB1 during the ensiling process of high moisture corn grains and to identify an endemic microbiota capable of degrading FB1. Laboratory scale silages were prepared with naturally contaminated FB1 grains from two cropping years. The analytical procedure allowed assessing both free and matrix associated FB1 forms. Results showed that whatever the initial amount of FB1 in corn grains, the concentration of matrix-associated FB1 did not change significantly while a significant decrease in free FB1 content occurred. It reached as much as 93% after 4 months of fermentation in certain silages. The stability of matrix-associated FB1 together with the detection of partially hydrolyzed FB1, called pHFB1, in some fermented samples, indicated that the free FB1 decrease was mainly due to degradation processes. Identification of a microbiota potentially responsible for this degradation was initiated. A metagenomics approach combining a selective microbial DNA extraction and high-throughput shotgun sequencing showed microbial distinct microbial diversity patterns between FB1 degrading and weakly degrading silage. Ultimately, this work brings evidence that high moisture grain ensiling can be a way to reduce FB1 contamination in feed by the presence/activity of a microbiota associated with FB1 biodegradation.

P82: Reduction of ergopeptine alkaloid concentrations in ergotized grasses by hay production

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Claviceps purpurea (ergot) is a fungal organism, portions of the life cycle of which infect many grasses, as well as small cereal grains. The vasoconstrictive and hypoprolactinemic properties of ergot alkaloids in the sclerotia of C. purpurea are responsible for the clinical signs of ergotism. Recently, the occurrence of atypically cooler environmental temperatures and greater than anticipated precipitation during the months of April, May, and June, have facilitated a greater incidence of C. purpurea-infected grasses in parts of the Midwestern United States. This increase in ergotized pasture grasses, including tall fescue (Lolium arundinaceum (Shreb.) S.J. Darbyshire) simultaneously infected with the ergot alkaloid-producing fungal endophyte, Epichloë coenophiala, has resulted in a dramatic increase in the incidence of serious adverse health effects attributable to ergotism in grazing cattle. The increasingly costly cattle production losses associated with C. purpurea in parts of the Midwest have stimulated greater interest in pasture management techniques which might help prevent ergotism. A preliminary experiment was conducted to determine how the physical manipulations of ergotized pasture grasses with mature sclerotia, during the mowing and baling processes, might affect the total concentrations of selected ergopeptide alkaloids. It was hypothesized that total concentrations of the measured ergopeptide alkaloids would be significantly less in hay samples, than in grass samples collected from each pasture prior to mowing and baling. Representative samples of grasses were collected from each of ten designated pastures in Missouri, and, within two weeks of baling, core samples were obtained from bales of hay from those pastures. The collected forage samples were submitted for analyses for six ergopeptide alkaloids (i.e., ergosine, ergotamine, ergocornine, ergocryptine, ergocristine, and ergovaline). The analyses were performed using high performance liquid chromatography with fluorescence detection. The Wilcoxon signed-ranked test for data without a normal distribution was used
for statistical analyses. The total concentrations of the ergopeptine alkaloids measured were significantly reduced ($p=0.002$) in hay samples, as compared to the corresponding grass samples. To conclude, while grass and hay samples will be collected and analyzed again this year, the results of this preliminary experiment demonstrate how producing hay from ergotized grasses with mature ergot sclerotia can reduce ergot alkaloid concentrations and help prevent ergotism.

**P83: Modeling support for minimizing aflatoxin B1 contamination in dairy cattle compound feed**

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Feeding dairy cattle with safe compound feed helps farmers to ensure food safety. However, several ingredients often used in compound feed production can be contaminated with aflatoxin B1 (AFB1), which may result into milk contaminated with aflatoxin M1. Given the number of ingredients and their amounts used in the production of compound feed, it is very costly to check every batch of ingredients for AFB1 contamination. That is the reason, a risk-based approach it taken in the latest years. This study aimed to estimate the probability of AFB1 contamination of compound feed for dairy cattle, and to limit this contamination, by optimization of the compound feed formulation, using a modelling approach. The modelling approach comprised integrating a linear optimization programming model to a Monte Carlo simulation model. This model was applied to the case of producing compound feed for dairy cattle in the Netherlands, using national monitoring data on AFB1 contamination in feed materials collected in the period 2000-2010. Results from this case study showed the model can be used to produce safe compound feed with the lowest possible probability of AFB1 contamination.

**P84: Inactivation of aflatoxigenic Aspergillus flavus by photosensitization with natural plant products**

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A study was conducted to investigate the photoactivation efficiency of curcumin (extract from turmeric) and Bidens pilosa leaf extract against Aspergillus flavus (a major aflatoxin producing fungus). Spores and hyphae of the fungus were subjected to controlled treatment combining plant extract photosensitizer treatment with exposure to light of specific intensity and wavelength. The effect of light was tested by varying intensity between 12 J/cm$^2$ and 84 J/cm$^2$ (420 nm wavelength) with photosensitizer concentration of 25-100 µM for curcumin and 0.25 mg/ml and 0.5 mg/ml for B. pilosa extract. The effect of temperature was studied by conducting the experiments between 15°C and 40°C. We observed a reduction in spore and hyphae by up to 4 logs, measured as colony forming units as a result of photosensitization treatment with either curcumin or B. pilosa. The results also indicated reduction of up to 2 logs of spores on surface of corn kernels with curcumin. The reductions were statistically significant ($p<0.05$) when compared to control groups. Magnitudes of spore and hyphae inactivation were influenced by photosensitizer concentration, light intensity and temperature. The results reveal the potential of natural photosensitizers derived from locally available plant material for post-harvest control of mycotoxigenic fungi in foods and feeds. This could be applied sustainably, especially in tropical and sub-tropical developing countries where mycotoxin contamination is a serious issue of concern in food safety.

**P85: Resistance of mycotoxins and fungi to commercial processing in apple and tomato products**

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Patulin and the *Alternaria* mycotoxins, alternariol (AOH) and alternariol monomethyl ether (AME) are toxic secondary metabolites known to be produced by fungi in both apples and tomatoes. Processing methods such as the removal of infected tissue and heat treatments are done to destroy both the toxins and the fungi. Research suggests that these methods may not be entirely effective and the possible co-occurrence of these toxins has not been investigated. This study examined the prevalence of fungi and
mycotoxins in food products following commercial processing. A variety of commercial apple and tomato products were tested for the occurrence of patulin, AOH, and AME with a solid phase extraction method. The same food products were then assessed for the presence of fungi that survived processing. This was accomplished by plating samples on malt extract agar and incubating for 14 days. The ability of these fungi to produce mycotoxins was not assessed. It was found that many commercial products contain some level of patulin, AOH, and/or AME. Patulin is the only one of the three toxins which is currently regulated, in Canada this limit is 50 µg/l. In some cases, patulin was found in levels exceeding regulatory limits. Fungi were found to grow from some samples of the tested tomato and apple products, showing a resistance to processing treatments. These results suggest that the current processing methods of fruit and vegetable products may be insufficient to protect against mycotoxins. The potential resistance of fungi presents the risk of mycotoxin production in foods if unsafe handling of the product occurs after processing.

P86: Critical point sources of ochratoxin A contamination in on-farm stored winter wheat

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A multi-year annual survey from 2011 to 2014 of the occurrence of *Penicillium verrucosum* and ochratoxin A (OTA) targeting on-farm stored winter wheat was conducted in 6 counties where approximately 50% of the total winter wheat grain in Ontario is produced. A total of 229 grain samples from 90 storage bins belonging to 61 farms were classified according to sample type (static or moving grain) and timing (before, during and after bin out-loading) into 6 categories: samples from untouched surface, disturbed surface, first load, intermediate loads, last load and grain clumps. In this survey, only 5 grain samples from 5 independent bins tested positive for OTA comprising 2.2% of the total samples taken and furthermore, only 3 samples, comprising 1.3% of the total grain samples taken, had OTA concentrations exceeding the proposed Health Canada guideline of 5 ng/g for raw cereal grains. However, a robust database was generated to identify critical points to minimize OTA accumulation in storage bins by adding in the floral data. Samples from grain clumps contained the most *P. verrucosum* (p<0.05, n=10, mean=5,447 CFU/g) followed by those taken from the first load (n=24, mean=147 CFU/g) or last load (n=17, mean=101 CFU/g) augered out of the bins, which was greater than those than from untouched (n=83, mean=81 CFU/g) and disturbed (n=16, mean=13 CFU/g) grain surfaces or intermediate loads (n=20, mean=9 CFU/g). When OTA production by isolates of *P. verrucosum* from soil and stored grain was determined from 1 day-old cultures, isolates from grain produced much higher quantities of OTA (115.4±16.1 µg/g) than isolates taken from the soil in which the grain was cultivated (11.4±6.4 µg/g). In storage, the distribution of *P. verrucosum* was highly heterogeneous, occurring in hot spots. Hot spots were most often in areas of the bin affected by moisture migration due to inadequate aeration and exposure to moisture from precipitation or condensation. Critical control points identified in decreasing order of importance were: (1) debris accumulated around access openings and door frames (100,991 CFU/g ±5,219; t=7.0; p<0.0001); (2) debris and residue of old grain left on storage floor (1,591 CFU/g ±825; t=5.4; p<0.0001); and (3) first and last grain loads during grain outloading (581 CFU/g ±254; t=4.6; p<0.0001). A summary of best management practices will be presented in this poster.

P87: Efficacy of a fungal and bacterial antagonist for controlling growth, FUM1 gene expression and fumonisin B1 by *Fusarium verticillioides* on corn cobs of different ripening stages

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Previous studies identified two biocontrol agents (*Clonostachys rosea* 016; Gram negative bacterium) with potential for control of fumonisin B1 (FB1) production in vitro and in stored corn under different water availabilities (Samsudin and Magan, 2016). These have now been complemented by studies of the efficacy of these two antagonists on corn cobs of different ripening stages: R3, Milk (0.985 aw); R4, Dough (0.976 aw); R5, Dent (0.958 aw). The cobs were inoculated with 50:50 mixtures of the pathogen:antagonist inoculum ratio and stored in environmental chambers to maintain these conditions for 10 days at 25 and 30°C. The growth rate of *Fusarium verticillioides*, the relative expression of the FUM1 gene and fumonisin B1 (FB1) production were quantified. Water activity (aw) x temperature had significant impacts on growth, FUM1 gene expression and FB1 production by the strain of *F. verticillioides* on corn cobs of different maturities. The *C. rosea* 016 antagonist significantly reduced FB1 contamination on corn cobs by >70% at 25°C, and almost 60% at 30°C regardless of corn ripening stage.
stage. For the bacterial antagonist FB1 levels on corn cobs were significantly decreased in some treatments only. These results suggest that efficacy of antagonists to control mycotoxin production in ripening corn cobs needs to take account of the ecophysiology of the pathogen and the antagonist to ensure that effective control can be achieved.

P88: Pre- and post-harvest management practices for minimization of mycotoxin contamination: a baseline study of knowledge, attitude and practices of subsistence farmers in Shamva and Makoni districts, Zimbabwe

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Aflatoxins pose a serious problem to corn grain rendering them unsuitable for human and livestock production. Presence of aflatoxins can be reduced through various strategies including improved pre- and post-harvest handling practices. In this study knowledge, attitude and practices were assessed with regard to good pre and post-harvest management practices that minimize mycotoxins contamination in corn and legumes. A total of 382 households were interviewed using a semi-structured questionnaire. Analysis on the farming method and the extent of mold attack in the field revealed that 44% of the farmers who practiced conservative agriculture experience mold attack, 39% of the farmers in practicing conventional farming encountered mold attack when the crop was in the field. Other researches have shown that no-tillage system increases the frequency of fungal spores. Of the households that witnessed pest/insect attack, 60.6% did not do anything to mitigate the attack. Households indicated that weed control is mainly done by hand (67.5%). Farmers acknowledged that they also encounter insect/pest problems during corn growth. Both pest and diseases can be sources of aflatoxin contamination on grain if no control measures are taken at field level. Households that dried their corn on the thatched-walled crib were 30.4%, 24.3% on bare floor and 22.8% on the wire-meshed walled crib. Analysis showed that 79% of the households that dried their cobs on the wire-meshed walled crib observed some molds during drying. In legumes, 42% were dried on a sack spread over the ground whilst 36.1% dried on bare floor. The major shelling method for legumes was hand shelling (61.1%) whilst for corn was rubbing on the stone (48.4%) and hand shelling (35.6%). Methods like beating or rubbing on the stone will lead to grain damage thus leaving it prone to disease attack. Some households further dried their grain after shelling (27.5%). Before storing the crop, 64.1% of the households assessed the moisture content of the crop. Most households (64.9%) sorted their grain according to variety, size and quality prior to storage whilst 69.9% sorted their legume according to variety, safety and quality prior to storage. The unhealthy grain sorted out would be used for animal fodder (80%), 17.3% would be discarded and 2.3% would be immediately cooked. The results show that some farmers have poor pre- and post-harvest management practices; hence there is need to train subsistence farmers on good farming practices that can minimize mycotoxin contamination of their crops.

P89: Use of soil bacteria in biological control against aflatoxins: the AFLAFREE project

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The main objective of AFLAFREE project (ANR 11 ALID 003 01) was to validate the proof of concept of the use of soil borne bacteria able to reduce aflatoxins content in vitro. The idea was to consider this system as a potential bacterial biocontrol efficient against the aflatoxin contamination in corn crop. Three main steps were conducted: (1) screening of Actinobacteria; (2) mode of action characterization and toxicity evaluation; and (3) conducting greenhouse experiments. To select this biocontrol, we made the hypothesis to focus on Actinomycetes strains (Streptomyces sp.) having a mutual antagonism in contact with Aspergillus flavus, i.e., promotion of both microorganisms’ growth, without impacting the natural microbial ecosystem in corn crop. Thus, the bacterial strains screening was performed in Petri dishes with Actinobacteria and fungus co-inoculation. The latter led to the discovery of many Actinomycetes strains able to reduce aflatoxins more than 90% in comparison with the control (Verheecke et al., 2014). We investigated their mechanism of action while microbial interaction. Six strains Streptomycyes were chosen. The expression of structure and regulator genes was either lower or higher depending on Actinobacteria strains, leading to aflatoxins reduction production (Verheecke et al., 2015a). However, we do not have yet identified where the biosynthesis pathway is stopped. The reduction of aflatoxin content, through reduction and adsorption tests was also investigated. Some Streptomycyes sp. Strains
can reduce pure aflatoxin B1 content in vitro. But till now, it was not possible to detect any degradation product. Concerning adsorption investigation, none of the selected strains exerted this mode of action (Verheecke et al., 2015b). Concerning the greenhouse experiments, the main objective was to test the biocontrol agent in corn plants. Three consecutive trials were conducted. 17 days after flowering, we carried out artificial inoculations of Actinomycetes strains. The bioaggressor A. flavus was inoculated with 2 modalities: jointly the same day or 7 days later. In these two modalities, one of our strains got a very significant aflatoxin B1 decrease of 50% on inoculated kernels. These promising results encourage us to continue the selection of efficient strains prior to field experiments. Acknowledgements. ANR Aflafree 2011-2015 N°11 003 01, Agrimip Sud Ouest Innovation.

P90: Critical control point-based mitigation of Fusarium mycotoxin production in stored grains of Job’s tears (Coix lachryma-jobi L.)

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Improperly practiced postharvest procedures can pose mycotoxin-related risks in the production of medicinal herbs. As a health food with pharmacological supplements, cereal-based Job’s tears has been broadly used in oriental medical practice. Compared with the standard production protocol, three provisional critical control points (CCPs) in the conventional procedure were identified and assessed for mycotoxin contamination in the Job’s tears from small farms in Korea. Although various mycotoxins were present, the prevalence of deoxynivalenol (DON) or zearalenone (ZEN) was relatively high in the Job’s tears. In terms of drying conditions, field drying in the conventional pathway was associated with more exposure to DON than heated-air drying. Moreover, the DON or ZEN levels in chaff were higher than the levels in the inner grain, suggesting that the hulling process as another CCP would reduce the DON or ZEN exposure. In particular, the DON or ZEN levels in Job’s tears stored for protracted periods without dehulling were very high, but a lower storage temperature of 12°C was not effective at significantly reducing these mycotoxins. In this case, the inner grain was more contaminated with DON or ZEN than the chaff after protracted storage because surface fungi, which produce mycotoxins, can penetrate deep into grain with time. Heated-air drying and non-protracted storage limited DON contamination in Job’s tears. More importantly, an early dehulling process should be adopted as an easy preventive action to reduce the risk of exposure to DON or ZEN in postharvest of Job’s tears. This is monitored as a central CCP for safer production of Job’s tears from local farms. Acknowledgements. This work was carried out with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01093206), Rural Development Administration, Republic of Korea.

P91: Effect of cropping measures on occurrence of Fusarium species and mycotoxins in grain corn – results from a multi-year survey in Switzerland

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In Switzerland, grain corn is grown on about 17,000 ha, mainly for animal feeding. Corn is frequently infected by a complex of Fusarium species causing root, ear and stem rot, resulting in substantial yield losses and reduced seed quality. More important, kernels are frequently contaminated with mycotoxins, which can cause severe animal health problems as some of them are carcinogenic, impair the immune system or reduce the fertility. To assess the risk of Fusarium and mycotoxin contamination in Swiss grain corn production, a survey was conducted during three years (2008-2010). Harvest samples from commercial fields and information on cropping measures were collected (n=288). Corn grains were examined for incidence of Fusarium species and mycotoxin content and the influence of different cropping measures was analyzed. A high variability of Fusarium species was found under which Fusarium graminearum, F. verticillioides, F. proliferatum and F. subglutinans were the most frequent species. However, their prevalence varied substantially from year to year. Deoxynivalenol (DON) was the most prevailing mycotoxin and in 51% of all samples, the European guidance value for swine feed of 0.9 mg/kg DON (complementary and complete feeding stuff) was exceeded. Levels of zearalenone
and fumonisins (FUM) were substantially lower and FUM were mainly found in samples of the canton Ticino, South of the Alps. Based on these results, we focused on identifying cropping measures crucial for FG infection and DON contamination. The date of harvest showed the greatest impact: samples harvested at the end of October or later contained up to six-fold higher DON contents than samples harvested at mid or end of September. Hybrids of mid-late maturity class had higher DON contents than hybrids of early or mid-early maturing class. This result was confirmed by analyzing a reduced dataset (n=115) of the survey, including only the four most commonly grown corn hybrids. Furthermore, reduced tillage significantly increased infection and mycotoxin contamination compared with samples from ploughed fields, irrespective of the previous crop. Cultivating cereals or corn as pre-previous crop significantly increased the risk of DON contamination in samples from ploughed fields. Since effects of hybrids and pre-crops might have been masked by the highly variable cropping conditions in our dataset, we examined corn grain samples from Agroscope variety trials (2011-2013). The analyses of this dataset demonstrated that samples from fields with previous crop corn increased FG infection and subsequent DON content. More importantly though, we were able to reveal substantial differences in hybrids with DON mean values ranging from 0.6 to 1.9 mg/kg, irrespective of the maturity class.

P92: Fate of enniatins and deoxynivalenol during pasta cooking
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The fate of mycotoxins during processing can improve the interpretation of data on occurrence of mycotoxins in raw materials for risk assessment purposes. The aim of this study was to assess the influence of cooking dry pasta on the occurrence of enniatins and deoxynivalenol in the end product. Twenty-six dry pasta samples were obtained from retail stores in the Netherlands in 2014 and were analyzed for 34 mycotoxins using an LC-MS/MS based method. Enniatins were detected in 73%, and deoxynivalenol in 15% of the dry pasta samples. The five dry pasta samples with the highest enniatins and deoxynivalenol levels (three spaghetti, one farfalle and one macaroni) were used for the cooking experiments. The five samples plus one replicate for spaghetti (to test repeatability) were cooked on one day in a laboratory setting. The experiments were repeated on a second, non-consecutive, day. The samples were cooked (100 g to 500 ml tap water) for 10 min and rinsed with water according to a pre-set protocol. The cooked pasta, the cooking water and the cooked pasta after rinsing were sampled. About 83-100% of the enniatins and 60% of the deoxynivalenol were retained in the cooked pasta samples. The findings emphasize the importance of increasing the knowledge on the fate of mycotoxins during food processing and food preparation for risk assessment. It is strongly advised to use naturally contaminated materials (incurred materials) for the food processing and food preparation experiments.

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P93: Comparing different methods of field application of bacterial endophytes as biocontrol agents to combat Gibberella ear rot in corn
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Fusarium graminearum Schwabe (teleomorph Gibberella zeae (Schweinitz) Petch) is a serious disease of wheat and corn found globally. Infection of the grain occurs during anthesis and silking respectively however infection of reproductive structures may also result from bird or insect feeding. Transmission to susceptible structures is typically achieved via asexual macroconidia or sexual ascospores. Infection reduces yield, however of greater concern and economic importance are the mycotoxins which are produced. Deoxynivalenol, also known as vomitoxin, is a trichothecene compound responsible for; gastrointestinal inflammation, vomiting, diarrhea, fever and abdominal pain. Outbreaks of symptoms have occurred in people consuming contaminated grain which has led to recommendations of no more than 1 mg/kg for human consumption. In livestock the toxin leads to lower feeding rates, higher veterinary costs and longer days to market. Zearalenone is an estrogenic metabolite which has been implicated in livestock reproductive disorders such as false pregnancy and spontaneous abortion. Endophytes are bacteria capable of living within plant tissues without causing disease symptoms. Some of these bacteria have demonstrated beneficial traits such as improved nutrient acquisition, disease suppression or growth promotion. Our lab previously screened the endophytic communities of several wild and ancient relatives of modern corn as well as the ancient Afro-Indian crop finger millet and found several strains of bacteria that were capable of actively antagonizing F. graminearum. These results
were confirmed first in vitro and subsequently with in vivo trials under greenhouse conditions. Current research focused on taking these bacteria to the field to explore their potential as biological control agents. Biological controls have well documented difficulties under the diverse and unpredictable conditions found in the field. This research aimed to examine the efficacy of four field application techniques when applied to five species of biocontrol endophytes. Of the possibilities, research was focused on strategies which could be most easily incorporated into existing cultural practices: seed coating, broadcasting of bacterial beads, spraying at silk emergence, and spraying at silk senescence. Resulting ears from these experimental plots were visually scored for disease severity and then grain mycotoxin quantities determined using liquid chromatography mass spectrometry.

P94: Biological control of ear rot (Aspergillus flavus) on sweet corn using Trichoderma harzianum

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One of the most promising management tools to reduce and manage mycotoxins in food and feed is the pre-harvest biological control of mycotoxigenic fungi using microbes. The goal of this investigation was to evaluate the potential of Trichoderma harzianum for control of Aspergillus flavus on sweet corn under field conditions. T. harzianum (strain KD) was applied as a pre-harvest spray treatment to silks of sweet corn plants at 1, 3, 6, 10, 12 and 14 days post-midsilk. Toxigenic A. flavus was spray inoculated as a conidial suspension (103 spores/ml) onto silks at 2, 4, 7, 9, 11 and 13 days post-midsilk. At physiological maturity, all ears were harvested, dried at 40°C for 7 days and shelled. Percentage kernel infection, disease ratings and high-performance liquid chromatography analyses was used to identify and quantify aflatoxin contamination. Preliminary results have shown a significant reduction of toxigenic A. flavus infection and aflatoxin contamination with T. harzianum silk spray treatments at 8 and 10 days post-midsilk. Ultrastructural studies using environmental scanning electron microscopy revealed that mycoparasitism is the probable mode of action. Initial signs of mycoparasitism were observed where hyphae of T. harzianum were seen coiling around A. flavus hyphae resulting in lysis and cell wall degradation. It thus can be seen that pre-harvest spray treatment of sweet corn at the silk growth stage can reduce the level of A. flavus contamination of grain. Results have spurred further evaluations into the pre-harvest biological control of Fusarium verticillioides and F. graminearum on corn using T. harzianum and natural yeast antagonists.

FACTORS AFFECTING TOXIN FORMATION

P95 – P106

P95: What impact will climate change scenarios have on aflatoxin contamination of pistachio nuts?

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Pistachio nuts are very commonly colonized by spoilage mycobiota especially aflatoxigenic species because they are very hygroscopic and can adsorb water. Aspergillus flavus can contaminate pistachio nuts under warm and humid conditions and contaminate the nuts with aflatoxins, classified as a class IA carcinogen. In many countries there is strict legislation on the maximum allowable limits for aflatoxins. There have been no studies on the impact that interacting climate change (CC) environmental conditions may have on growth of A. flavus and aflatoxin B1 (AFB1) production on pistachio nuts. Thus the objectives of this study were to examine the effect of CC interacting factors of temperature (35 and 37°C) x water activity (aw, 0.93, 0.95 and 0.98) x CO2 (350 and 1000 ppm) on (1) growth of A. flavus strains (AB3, AB10) isolated from pistachio nuts, (2) the effect of CC factors on relative genes expression of the aflD and aflR genes involved in the biosynthetic pathway for AFB1 production, and (3) AFB1 production in pistachio nut-based media and in pistachio nuts. These studies showed that the effect of interacting CC factors on growth of A. flavus colonization was not significant. However, AFB1 production was stimulated. With regards to aflD gene expression, at 35°C, the relative expression was higher at 350 ppm CO2 for both strains except for AB3 where the expression was higher at 1000 ppm CO2 at 0.95 aw. However, at 37°C, the expression was generally higher at 1000 ppm CO2 than with existing atmospheric CO2 levels. The aflR gene expression was higher at 1000 ppm CO2 at 37°C for both strains. AFB1 production was higher at 35°C at the two CO2 levels for both strains. At the same
temperature, AFB1 production was significantly increased at 1000 ppm CO2 and 0.98 aw. At 37°C, AFB1 production was either decreased in strain AB3 or similar as in strain AB10 when exposed to 1000 ppm CO2. This suggests that CC factors may have a differential effect depending on the interacting conditions of temperature (35 or 37°C) as in some cases AFB1 production was stimulated while in others remained the same. Studies are in progress to examine whether acclimatization to elevated CO2 of A. flavus strains affects AFB1 production under interacting CC conditions and whether this is different from non-acclimatized culture (Median et al., 2014; 2015a,b).

P96: The impact of climate change on free and masked fumonisins

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Fumonisins are a group of mycotoxins frequently found in corn worldwide, mainly associated to Fusarium verticillioides. Although a large number of fumonisins have been identified, the B group (i.e., FB1 to FB4) is the most prevalent in food and feed commodities. Starting from the observation that alkaline hydrolysis of contaminated corn products leads to a higher amount of released FBs than expected, studies on the possible occurrence of modified forms have been performed over the last decade. A number of unidentified bound forms were detected in thermally treated food products. However, the so called ‘hidden fumonisins’ have been proven to occur in corn kernels harvested in commercial fields. The mechanism of FB modification is still an issue. The parent compounds can be physically entrapped into the structure of macromolecular components, such as starch or proteins, or the hydroxyl groups and the amino groups are involved in the formation of fatty acid conjugates in plants. The only conjugated forms of FB that have been isolated in naturally infected kernels so far are due to the esterification of FBs with oleic and linoleic acids. Corn hybrids, water activity and amylose/amylpectin ratio in grain have been shown to affect the extent of masking, which is however mostly related to lipid compounds. As these fatty acids are precursors of oxylipins, the correlation of these compounds with mycotoxins underlines that the plant-fungus cross talk strongly affects FB modification. In climate change scenarios, relevant stress will occur both in crops and mycoflora. F. verticillioides maximizes FB production at marginal aw around 0.95, this will be surely affected in warmer seasons. CO2 increase impact on corn susceptibility to F. verticillioides, because of differences in accumulation of some compounds, i.e., sugars, free fatty acids, lipoxygenase (LOX) transcripts. Elevated CO2 seems to compromise cor LOX dependent signaling and, based on available knowledge, this is expected to affect also FB modification. Research efforts should focus in the next future on the events occurring in field and on the cross-talk between the plant and the fungus, to fill the lack of knowledge, mainly regarding fumonisin masking, to provide useful data for mitigation of fumonisin free and masked forms, for predictive models, the most suitable to forecast what is going to happens in the future changing climate, and to support human exposure assessment, also in account of hidden fumonisins.

P97: Fungi and mycotoxins in cheese – modeling the ecological needs

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Aspergillus and Penicillium fungi can grow on cheese and contribute to the characteristics of the final product; however, sometimes they have a negative impact on cheese quality and safety. Recently, ochratoxin A (OTA), sterigmatocystin (STC), roquefortine C (ROQ-C), mycophenolic acid (MPA), citrinin (CIT) and cyclopiazonic acid (CPA) have been detected in cheese. Although OTA and STC are mycotoxins included in group 2B (possibly carcinogenic to humans) by IARC (1993; 1987), they are not included in EU regulation. To date, the role of ecological factors on the activity of these fungi is poorly studied. The aim of the present study was to (1) define the ecological needs (temperature, T; water activity, aw) of eight fungal species, including Aspergillus versicolor, Penicillium camemberti, P. citrinum, P. crustosum, P. nalgiovense, P. nordicum, P. verrucosum, and P. roqueforti, and assess their ability to produce mycotoxins (STC, CPA, CIT, OTA, ROQ-C, MPA); and (2) to describe by mathematical models fungal activity in different T and aw regimes. Fungal growth was studied in vitro on artificial substrate (Czapek Yeast Agar, CYA) with T ranging from 5°C to 40°C (step 5°C; aw=0.99) and aw, ranging from 0.87 to 0.99, (step 0.03; T=20°C). After 3, 7, 10 and 14 days of incubation, colony diameters and after 14 days mycotoxins production were measured. All fungi were able to grow between 5 and 35°C, except A. versicolor and P. camemberti, with a useful range 10-30°C and 5-25°C, respectively. The optimal temperature for growth was 25°C, except for P. citrinum (30°C), P. nordicum and P. verrucosum.
(20°C). Regarding aw, the growth range was between 0.87 and 0.99. A. versicolor and P. roqueforti grew from 0.87 aw, while aw=0.90 was the minimum for P. citrinum, P. crustosum, P. naigiovense, and P. verrucosum. The optimal aw for growth was 0.99, except for A. versicolor, with aw=0.96. Fungal growth was described by (1) Betes functions of Analysys for T and (2) logistic equations for aw regimes. For mycotoxins, the optimal production was in the range 15-25°C, except for A. versicolor (optimum for STC, 30°C) and P. citrinum (optimum for CIT, 35°C). Regarding aw, the optimal production was at aw=0.99 for all species and toxins (except for MPA, aw=0.96). These data are a good basis to predict the risk of mycotoxin contamination in cheese related to environmental/storage conditions. Evidence will be confirmed by further studies using cheese as a substrate.

P98: rtfA, a putative RNA-pol II transcription elongation factor gene, is necessary for normal morphological and chemical development, proper response to oxidative stress and pathogenicity in Aspergillus flavus

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The agriculturally important opportunistic plant pathogen Aspergillus flavus produces potent carcinogenic compounds called aflatoxins. We identified the A. flavus rtfA, ortholog of rtf1 in S. cerevisiae and rtfA in A. nidulans. rtfA has multiple cellular roles in this aflatoxin-producing fungus. In this study, we show that rtfA regulates vegetative growth, conidiation and sclerotial production. Additionally, the absence of rtfA notably reduces aflatoxin B1 production, indicating that rtfA is a positive regulator of mycotoxin biosynthesis in A. flavus. Furthermore, rtfA also influences the synthesis of other unknown metabolites. Interestingly, rtfA regulates the expression of veA and laeA, global regulators known to control secondary metabolism and morphogenesis. Importantly, rtfA is also required for normal pathogenicity in plant (peanut seed) and animal (Galleria mellonella) models. A greater sensitivity to oxidative stress; decrease in protease and lipase activity and a decrease in biofilm formation in the rtfA deletion mutant might contribute to the observed decrease in virulence.

P99: Impact of climate change factors on growth and ochratoxin A production by Aspergillus section Circumdati and Nigri species on coffee

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The objectives were to evaluate the effect of interacting climate change (CC) conditions (water stress (water activity, aw; 0.99-0.95); temperature (30, 35°C); and elevated CO₂ (400 and 1000 ppm)) on: (1) lag phases prior to growth; (2) growth; and (3) ochratoxin A (OTA) production by species of Aspergillus sections Circumdati and Nigri on coffee-based media and stored coffee beans. The lag phases, prior to growth, of all strains/species were slightly increased as aw, temperature and CO₂ were modified. The interacting CC factors showed that most strains/species examined grew well at 30°C and slightly less so at 35°C except for A. niger (A 1911) that could tolerate the higher temperature. In addition, the interaction of elevated CO₂ (1000 ppm) + increased temperature (35°C) increased OTA production when compared with 30°C for strains of A. westerdijkiae (B 2), A. ochraceus (ITAL 14) and A. steynii (CBS 112814). Most of the strains had optimum growth at 0.95 aw, at 35°C while, at 30°C the optimum was at 0.98 aw. On coffee beans there was a significant stimulation of OTA production by A. westerdijkiae strains in elevated CO₂ (1000 ppm) at 0.90 aw. However, overall there were differential effects of CC factors on OTA production by species in the Sections Circumdati and Nigri in coffee. This could have significant implications for quality of coffee used for different consumer products.

P100: Identification and classification of agronomic factors involved in ergot and its alkaloids content in small grain of cereals

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The European Commission Regulation (EU) 2015/1940 of 28 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia in certain unprocessed cereals establishes maximum limits for ergot content (Claviceps purpurea) in small grain cereals for human consumption. Occurrence studies have shown that all the small grains cereals are affected by this disease, triticale
and rye are the most sensitive. Since 2012, ARVALIS - Institut du végétal studied more than 2,000 farm fields of common wheat, durum wheat, barley, rye and triticale according to the same methodology, in collaboration with partners. This work allowed one hand to determine the relationship between ergot contamination of crops, and production of associated alkaloids. The total average alkaloid content is 0.32% varying from 57 to 36,385 µg/g ergot bodies, which means from 0.006 to 3.6% of ergot bodies weight. The ratio between -ine and -inine forms is 2.6:1. Ergotamine, ergocristine, ergosine and their corresponding epimers represent 74% of total alkaloids. The mean of total alkaloids content of C. purpurea is 3,103 µg/g. The host plant and the harvest year did not have any influence on the alkaloid content. The ergot content explains 79% of the variability of alkaloid content. On the other hand, the survey led to identify and prioritize agronomic drivers to limit the risk in crops. A statistical analysis was applied on ln(total alkaloid). The variance analysis applied to agronomic factors showed that the host plant, the previous crop, grassweeds presence and tillage system significantly influence alkaloids content. The relevance of these drivers was confirmed by analytical testing. This work is a multifactor field prevention tool to limit and manage the ergot and alkaloids risk. Nevertheless, we know that the main factor involved in ergot infection is the weather conditions.

**P101: Fungal biotransformation of chlorogenic and caffeic acids by Fusarium graminearum – new insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals**

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*Fusarium* head blight (FHB) and *Gibberella* ear rot (GER), mainly caused by the fungi *Fusarium graminearum* and *F. culmorum*, are devastating diseases of small-grain cereals and corn. In addition to yield loss, these diseases frequently result in contamination of kernels with toxic type B trichothecenes (TCTB). The breeding of tolerant genotypes is one of the most promising strategies to efficiently control mycotoxins in cereals. We recently demonstrated that chlorogenic acid could play an important role in resistance of cereals to TCTB-producing fungi and TCTB accumulation. In the present study, the effects of chlorogenic acid and one of its hydrolyzed products, caffeic acid, on fungal growth and TCTB biosynthesis were investigated. Concentrations close to physiological amounts quantified in kernels and a set of *F. graminearum* and *F. culmorum* strains were used. Both chlorogenic and caffeic acids were shown to negatively impact fungal growth and mycotoxin production, with caffeic acid being significantly more toxic. To decipher the mechanisms involved in the antifungal and ‘antimycotoxin’ efficiency of chlorogenic and caffeic acids, the metabolic fate of these two phenolic acids was characterized in supplemented *F. graminearum* broths. For the first time, our results demonstrated the ability of *F. graminearum* to degrade chlorogenic acid into caffeic, hydroxycchlorogenic and protocatechuic acids and caffeic acid into protocatechuic and hydroxy caffeic acids and indicated that some of these metabolic products can contribute to the inhibitory efficiency of chlorogenic acid. Chlorogenic acid can therefore be compared as a ‘pro-drug’. As a whole, our data corroborate the contribution of chlorogenic acid to the chemical defense that cereals employ to counteract *F. graminearum* and its production of mycotoxins.

**P102: The functional characterization of rmtA, an arginine methyltransferase in the plant pathogen Aspergillus flavus**

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The genus *Aspergillus* is a group of filamentous, saprophytic fungi that includes some of the most beneficial and detrimental fungal species. Among them, *Aspergillus flavus* is a well-known opportunistic pathogen often found colonizing our food commodities, particularly important oil seed crops, contaminating them with mycotoxins, such as aflatoxins. In order to gain insight into the genetic regulatory mechanisms controlling *A. flavus* mycotoxin production, as well as dissemination and survival, in this study we characterized rmtA, a gene encoding a putative type I arginine methyltransferase involved in histone modifications. Our work has revealed that aflatoxin production is significantly decreased in the rmtA deletion mutant (ΔrmtA) and greater in the over-expression strain compared to the wild-type, indicating that rmtA is a positive regulator of aflatoxin biosynthesis. Further studies indicated that the effect of rmtA on aflatoxin production is mediated by aflJ and aflR. Interestingly, transcriptome analysis has shown that, in addition to the aflatoxin gene cluster, several other secondary
metabolite gene clusters are regulated by rmtA, including the aflatrem and cyclopiazonic acid clusters. The ΔrmtA colonies also presented hypercondiation, suggesting that rmtA is a repressor of asexual development in A. flavus by regulating the brlA-abaA-wetA pathway. In addition, sclerotial development is also rmtA-dependent. In the absence of rmtA sclerotial production is reduced or prevented, while forced over-expression of rmtA results in an increase in the formation of these resistant structures. Furthermore, our studies show that rmtA and the global regulator veA are functionally connected.

P103: Relationship between ochratoxin A accumulation and pathogenicity of Aspergillus spp. in grapes

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Many fungi are epiphytic on grapes in the vineyard, but the main concern is the group of black mycotoxigenic Aspergillus species, in particular Aspergillus carbonarius, A. tubingensis and A. niger. These species are the causal fungal pathogens of black rot diseases in the field, and may cause severe postharvest fruit decay and accumulation of secondary metabolites such as the mycotoxin ochratoxin A (OTA) in the colonized tissue. A. carbonarius is considered the main agent responsible for OTA contamination in grapes because the proportion of OTA producing strains in this species is higher than those tested for members of the A. niger aggregate. The mechanism of OTA accumulation and its contribution to A. carbonarius pathogenicity remain unclear. Host pH modulation by pathogens has been suggested as an important factor in enhancing the virulence of postharvest pathogens. The secretion of pH modulating molecules such as D-gluconic acid (GLA) and ammonia by A. carbonarius was examined in order to investigate the role of these specific factors in an induction of OTA in vitro and in vivo. The growth of A. carbonarius in solid GLA inducing medium at initial pH 7.0 caused a rapid accumulation of GLA up to 17 mg/g mycelium dry weight (MDW) and a decrease in pH from 7.0 to 3.45, 3 days after inoculation. Ammonia accumulation increased gradually in culture starting on day 6 (after the initial GLA decrease), reaching 1185 μM at day 14 with a slight pH increase to 3.87. Under these conditions, OTA accumulation increased to over 2000 µg/kg 14 days after inoculation with a concomitant decrease in GLA (2.28 mg/g MDW at day 14). The similar pattern of A. carbonarius metabolites production has been observed in colonized tissue. The present data suggest that ambient pH is a regulatory signal for induction of mycotoxin production caused by Aspergillus spp. under the dynamic nutritional growth conditions occurring in culture. Yet, the molecular mechanisms inducing OTA biosynthesis during colonization of the host acidic environment are still unclear and should be further investigated.

P104: Patulin in ‘Galaxy’ apple – effect of different controlled atmosphere storage conditions and 1-MCP application

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Patulin is a mycotoxin that can especially be found in apple products. In Brazil, apple harvest begins in February and finishes in April. Therefore, a storage system is needed for fruit supply all year, mostly by applying controlled atmosphere (CA). Therefore, the aim of this study was to investigate patulin in ‘Galaxy’ apples after storage under different CA conditions. Apples harvested from commercial orchards in February 2015 in Vacaria, Rio Grande do Sul, Brazil, were subject of a completely randomized 2x3 factorial experiment, with in total 6 treatments with 4 replications. Factor A was presence and absence of 1-methylcyclopropene (1-MCP). Factor B was: (1) controlled atmosphere (CA) with 1.2 kPa O2 and 2.0 kPa CO2; (2) dynamic controlled atmosphere (DCA) with chlorophyll fluorescence measurement (DCA-CF); and (3) DCA with monitoring the respiratory quotient (DCA-RQ). All treatments were applied in experimental chambers at 2.0°C and a relative humidity of 96%. After 8 months storage the fruits were removed and exposed to 20.0°C for 7 days. Patulin was determined according to the method developed and validated by CEPARC/UFSM in healthy (fruits without any damage) and rotten apples (fruits which showed lesions with a diameter greater than 5 mm). Healthy fruits did not show any patulin in all treatments. In rotten apples, however, addition of 1-MCP increased the patulin concentration. In addition, there was no significant difference in patulin concentration between CA and DCA treatments, both with and without 1-MCP. Likewise, there was no significant difference between both DCA

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spoiled rice was analyzed, but none were above the FDA recommended limit (2 mg/kg). Food items acquired as wages or those grown on a villager’s own farm had significantly higher aflatoxin levels than food items acquired in formal market settings. Household food safety, waste management, and storage practices did not affect the presence and accumulation of aflatoxin in this survey. However, storage time did have a positive effect on aflatoxin levels in rice. Awareness of mycotoxins being a food safety issue was not associated with social class or aflatoxin accumulation. These preliminary results indicate that social and behavioral factors influence dynamics of mycotoxin accumulation in the Aurepalle food system. We hypothesize that mycotoxin risk is also dynamic over space and time, and would be magnified in the rainy season. Because rice is the dominant staple food in Aurepalle, it is plausible that rice consumption accounts for a large proportion of villagers’ exposure to aflatoxins.

P105: An in vitro experiment to determine the levels of ochratoxin a produced under controlled temperature and moisture conditions in wheat kernels


In Canada, ochratoxin A in cereal grain can be produced by Penicillium verrucosum, which develops after harvest during storage, if temperature and moisture conditions are conducive to mold growth. An in vitro experiment was designed to monitor the levels of ochratoxin A in single kernels of wheat over time. In each of the 34 glass test tubes, 1 inoculated (dyed red) wheat kernel was placed on top of 100 sterilized wheat kernels. The test tubes were placed into an incubator and kept constant at 20 degrees C in darkness and ~20% moisture content. Starting at time zero (t=0) to week 59 (t=33) a total of 3,434 samples were analyzed. From each test tube, all the kernels were individually weighed out and analyzed for ochratoxin A and B using a Waters Acquity UPLC with fluorescence detection. Run times of 5 minutes per sample allowed all 101 kernels per test tube to be analyzed in a single run that took approximately 12 h. Pictures were taken of the samples before analysis and at t=0, no signs of mold were visible and the only positive sample was the one inoculated kernel. At t=3 (3 weeks), there was signs of mold growth on a few of the kernels on top of the sample next to the inoculated kernel (4 positives). At t=7, there was visible mold (light greenish gray like material) on the entire sample. (76 positives, levels in the μg/kg range). At t=14 (14 weeks), the mold was becoming heavier over the sample (91 positives, a few in the mg/kg range). By t=20, mold continues to increase (100 positives, most in the mg/kg range).

P106: Food system-wide preliminary survey of factors associated with mycotoxin risk in Aurepalle, Telangana, India

Anthony J. Wenndt and R.J. Nelson

Aspergillus spp. and Fusarium spp. produce the mycotoxins aflatoxin and fumonisin, respectively. Mycotoxin contamination in food systems has serious implications for agricultural output and human health, especially in the developing world. In India, little is known about the spatial and temporal dynamics of village-level mycotoxin exposure risks. Here, we report on a food system-wide preliminary survey of factors associated with mycotoxin risk in Aurepalle village in Telangana, India, which was facilitated by ICRISAT and the Tata-Cornell Agriculture and Nutrition Initiative. We collected 293 samples of major food items including sorghum, corn, rice, pulses, coriander, and groundnut from 60 households, three marketplaces, two ration shops, four grain mills, and one anganwadi (maternal/child nutrition center) and measured aflatoxin and fumonisin with a digital lateral flow device immunochromatographic assay method. Aflatoxin was detected in 6.5% of household samples; among those samples, 35.7% exceeded the Indian legal limit (30 μg/kg). Fourteen percent of household rice samples had detectable aflatoxin, with 5.8% contaminated over the legal limit. Fumonisin was detected in 20% of all samples analyzed, but none were above the FDA-recommended limit (2 mg/kg). Food items acquired as wages or those grown on a villager’s own farm had significantly higher aflatoxin levels than food items acquired in formal market settings. Household food safety, waste management, and storage practices did not affect the presence and accumulation of aflatoxin in this survey. However, storage time did have a positive effect on aflatoxin levels in rice. Awareness of mycotoxins being a food safety issue was not associated with social class or aflatoxin accumulation. These preliminary results indicate that social and behavioral factors influence dynamics of mycotoxin accumulation in the Aurepalle food system. We hypothesize that mycotoxin risk is also dynamic over space and time, and would be magnified in the rainy season. Because rice is the dominant staple food in Aurepalle, it is plausible that rice consumption accounts for a large proportion of villagers’ exposure to aflatoxins.
P107: Effects of fingerroot extracts on aflatoxin B1 epoxide formation and mutagenicity

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The objective of this study was to investigate potential inhibitory effects of the culinary herb, fingerroot (Boesenbergia rotunda) on the formation of aflatoxin B1 epoxide (AFBO) and its mutagenicity. In vitro formation of AFBO from aflatoxin B1 (AFB1) was measured indirectly with rat microsomal membrane in the presence of NADPH. AFB1 dihydrodiol (AFB1-DHD), the hydrolysis product of AFBO, was employed as an indicator of AFBO and was detected by LC/MS and HPLC. Fingerroot was extracted with methanol (ME) and then partitioned with chloroform/water to obtain the organic (ME-O) and matching aqueous (ME-A) fractions. The extracts (ME, ME-O and ME-A) were analyzed by UV-Vis. spectrum scanning, HPLC and LC/MS. They showed Amax at 340 and 280 nm which corresponded to flavonoids. Six flavonoids were detected in ME and ME-O by HPLC, namely pinocembrin, pinocembrin chalcone, cardamonin, pinostrobin, panduratin A and 4-hydroxy panduratin A. The ME-A showed a comparatively low content of these compounds. Cardamonin (ME-CDN) was purified from ME-O and confirmed by LC/MS. The inhibitory effects of the ME, ME-O, ME-CDN and some flavonoid standards (apigenin, cardamonin and pinostrobin) were evaluated against AFBO formation in an in vitro metabolic system (Model I) and AFB1-mutagenicity in Ames test (Model II). Model I: Apigenin was the most potent inhibitor against AFB1-DHD formation (IC50=2.76 μM) followed by ME-O (IC50=6.61 μM) and the ME (IC50=6.87 μM) each of which maximal caused inhibition that approached 100%. No inhibition was found with the ME-A. Model II: All testing compounds reduced AFB1 mutagenicity to some extent. The most potent inhibitor against AFB1 mutagenic activity was the ME (7.34 μM). Whereas, the ME-O, apigenin and cardamonin standards showed comparable IC50 values (0.01-0.03 mM) which, however, were much lower than that of the ME. The ME-CDN and pinostrobin standard showed the lowest IC50 (0.1-0.8 mM). These observations suggest that AFBO is not the primary mutagen in the Ames system but that components of ME provide protection but the P450-inhibition study demonstrates that flavonoids in fingerroot block AFBO formation by altering aflatoxin metabolism at the initial stages of enzymatic activation and hence reduce AFB1 mutagenicity.

P108: Efficacy of UNIKE® Plus to reduce the toxicity of a combination of aflatoxin B1 and fumonisins in B1 in weaning pigs

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The objective of the study was to determine the efficacy of the mycotoxin deactivator UNIKE® Plus to prevent or reduce the toxic effects of the combination of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) in weaning pigs. On day 14 post-weaning, thirty 5-week-old pigs were assigned to each of 5 dietary treatments for 26 days. Dietary treatments evaluated included: (1) basal industry type weanling diet (BD) containing no mycotoxin deactivator or mycotoxins; (2) BD plus 0.50% UNIKE® Plus (UP); (3) BD plus mycotoxin contaminated diet – MM (1 mg/kg AFB1 and 25 mg/kg FB1); and (4) BD plus MM and 0.25% UP; and (5) BD plus MM and 0.50% UP. Pigs were weighed on day 26, euthanized and necropsied at the University of Missouri Veterinary Medical Diagnostic Laboratory. Feed intake, body weights, liver and kidney weight and liver lesion score were measured on day 26. Aflatoxin B1, M1 and G1 residues were measured in the liver and kidney. Effects of dietary treatments on serum biochemistry, total antioxidant capacity, lipid peroxides, and aqueous peroxides were measured in serum. UNIKE® Plus at 0.25% reduced the negative effects of mycotoxins on lipid peroxides, reduced AFB1 levels in liver by 36%, and improved serum total protein to control values in pigs fed dietary treatments for 26 days. However, the serum total protein value was not statistically different from that of pigs fed only mycotoxins. UNIKE® Plus at 0.50% reduced the negative effects of mycotoxins on feed intake, body weight gain, feed efficiency, lipid peroxides, improved serum total protein to control values, and significantly reduced AFM1 and AFB1 residues in the liver by 47% and 55%, respectively. In conclusion, UNIKE® Plus at 0.25% was only marginally effective in reducing some of the negative effects of mycotoxins. In contrast, UNIKE® Plus at 0.50% was more effective in reducing the negative effects of mycotoxins in weaning pigs. Finally, results indicate that 0.50% UNIKE® Plus did not cause any negative
Aflatoxins are a group of mycotoxins and secondary metabolites synthesized primarily by food-borne fungi such as *Aspergillus parasiticus* and *A. flavus*. Aflatoxin B1 is the most potent liver carcinogen known and its contamination in food and feed is a significant threat to public health and the economy. With liver carcinomas already being the third leading cause of cancer-related mortality worldwide, the global increase in prevalence of hepatitis B virus (HBV) and immunocompromised population has increased the risk of aflatoxin-induced liver cancer. The elimination of aflatoxin accumulation in food and feed, therefore, is of primary importance for reducing its global burden on public health and economy. Available approaches reported thus far have been insufficient to eliminate this threat, and over 55 billion people worldwide still suffer from uncontrolled exposure to aflatoxin, resulting in an est. 25,200 to 155,000 liver cancer cases globally and therefore provide the rational to explore novel methods for preventing aflatoxin accumulation in the environment. While many terrestrial plants and microbes that share ecological niches and encounter the aflatoxin producers have the ability to synthesize compounds that inhibit aflatoxin synthesis, reports of natural aflatoxin inhibitors from marine ecosystem components that do not share ecological niches with the aflatoxin producers are rare. Here we show that a non-pathogenic marine bacterium, *Vibrio gazogenes*, when exposed to low non-toxic doses of aflatoxin B1, demonstrates a shift in its metabolic output and synthesizes a metabolite fraction that inhibits aflatoxin synthesis without affecting hyphal growth in the model aflatoxin producer, *A. parasiticus*. The molecular mass of the predominant metabolite in this fraction was also different from the known prodigiosins, which are the known antifungal secondary metabolites synthesized by this *Vibrio*. Gene expression analyses using RT-PCR demonstrate that this metabolite fraction inhibits aflatoxin synthesis by down-regulating the expression of early-, middle- and late-growth stage aflatoxin genes, the aflatoxin pathway regulator, aflR and one global regulator of secondary metabolism, LaeA. Our study therefore establishes a novel system for generation of aflatoxin synthesis inhibitors, and emphasizes the potential of the under-explored *Vibrio*’s silent genome for generating new modulators of fungal secondary metabolism.

**P110: Biodegradability of mycotoxins in anaerobic digestion: a new solution to valorize contaminated mycotoxin batches?**

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Mycotoxins are toxic, low-molecular-weight, secondary metabolites produced by fungi. They occur in a wide variety of food and feed commodities, and are of major public health concern because they are the most hazardous of all food and feed contaminants in terms of chronic toxicity. To date, highly-mycotoxin contaminated food and feed batches are removed from the market by combustion, when exceeding maximum limits or guidance values (2003/100/EC; 2006/576/EC; 2013/185/EU). The Public Waste Agency of Flanders (OVAM) dictates the fate of waste streams and their appropriate removal. In relation to the valorization of organic biological waste (OBW), OVAM uses 60% of the total OBW-input in biogas digesters. In Flanders, 40 biogas plants are in operation or under construction with a total processing capacity of 2.234 million tons per year, of which 9 thermophilic and 31 mesophilic installations. When OBW is processed through anaerobic digestion, specific standards (VLAREMA) should be respected. Main consequences are that there are some standards for environmental contaminants to be imposed on the input materials for fermentation. Specifically for mycotoxins, OVAM rejects the processing of OBW due to insufficient guarantees about the degradation and the behavior of the mycotoxins in the fermentation process, and the subsequent marketing of the end products, such as fertilizers or soil improvers in the agricultural industry. This poster gives an overview of the biodegradability of aflatoxin B1, deoxynivalenol, zearalenone, fumonisin B1, ochratoxin A, T-2 toxin and ergot alkaloids in thermophilic and mesophilic digestion installations. Digestes were analyzed with a newly-developed LC-MS/MS procedure for the simultaneous determination of mycotoxins in aqueous matrices. Main
focus will be pointed out on the different steps of the method development using LC-MS/MS and the biodegradability capacity of bio-gas installations. Specific results will be extensively clarified, discussed, and extrapolated to the overall biogas industry. The obtained knowledge gleans crucial information to valorize mycotoxin-contaminated OBW-batches in biogas digestion installations, and proves that LC-MS/MS is the method of choice to verify the problematic nature of mycotoxins.

**P111: On the pursuit of mycotoxins degrading enzymes – a straightforward framework based on a joint in silico/in vitro approach**

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Nowadays the role of mycotoxins in affecting the human health and wellbeing is an indisputable fact, being contamination of food, feed and raw materials widespread worldwide. Besides the toxicological risk, their occurrence in food and feed commodities strongly affect the market in terms of global trade and food security. Although good agronomical practices and control strategies have been developed along the food/feed production chain, fungal infection and mycotoxin accumulation in field and during storage cannot be completely avoided. For this reason, the implementation of proper mitigation strategies to be applied along the food chain are actually a pivotal challenge for ensuring food/feed safety – also in view of the trade safeguard by recovering products otherwise off-market due to over-contamination. Among the proposed approaches, the enzymatic route seems to be the most promising. As a matter of fact, the search and engineering of effective enzymes for converting mycotoxins into nontoxic forms serve the cause doubly. On the one hand, enzymes can be optimized for application at various stages of food/feed chain (i.e. downstream mitigation). On the other hand, recombinant enzymes can be accounted for the expression in genetically modified organisms for preventing the accumulation of mycotoxins in plants (i.e., upstream mitigation). In this framework, our efforts have been devoted to apply hybrid in silico/in vitro approaches for: (1) profiling the array of metabolic forms that truly mediate toxic effects in the living organism beyond the parent compounds (including both plant and mammal metabolism); (2) identifying and engineering detoxifying enzymes for degrading such forms; and (3) assessing the lack of toxicity of biotransformed products. More in detail, predictive metabolism studies may extend the array of known plant and mammals metabolites. Then, the coarse-grained screening in silico of such metabolites (and biotransformed products as well) vs. the most relevant toxicological endpoints can drive the identification of (novel) bioactive forms. In their respect, the identification and optimization of detoxifying enzymes was pursued by screening database of enzymes including wild type forms from different organisms and combinatorial libraries. This poster will describe the possible application of this innovative approach to aflatoxins and zearalenone as case studies.

**P112: Anti-mycotoxin additives evaluated in animals**

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In vivo evaluations of 268 anti-mycotoxin additive products (AMA) were performed from January 2005 to December 2015 in different species: broiler, laying hen, turkey, Pekin duck, fish, pig and dairy cow. The used mycotoxins were aflatoxins, fumonisins, T-2 toxin and zearalenone, which were produced by toxigenic fungal strains grown on corn. The experimental designs were formed according to the addition of AMA and/or mycotoxins to the diet, with a minimum of 4 treatments: (1) control (diet free of mycotoxins or AMA); (2) diet containing AMA; (3) diet containing mycotoxins; and (4) diet containing mycotoxins and AMA. The diets were prepared in accordance with the nutritional requirements of the species and addition of AMA ranged from 0.15 to 0.5%. The animals were allocated under environmental conditions, handled according to the species requirements and food and water were available ad libitum throughout the experimental period, which lasted between 21 and 48 days. The most frequently evaluated parameters were the zootechnical data (such as feed consumption, weight gain and feed conversion), relative weight and size of target organs, TPT sphingolipids and aflatoxin M1 in dairy cows. The data obtained in the experiments were subjected to analysis of variance and Tukey’s or Bonferroni test were then applied (p ≤ 0.05). AMA was considered efficient when the relevant parameters presented significant difference. Results of the tested products for each species, number of evaluated AMA and % of approvals were: broiler – aflatoxins, 82 (54.3%); fumonisins, 48 (39.6%); T-2 toxin, 4 (0%); and aflatoxins+fumonisins+T-2 toxin, 14 (57.1%); laying hen – aflatoxins, 1 (100%); aflatoxins+fumonisins+...
Partial effectiveness of a detoxifying agent to prevent deleterious effects of dietary zearalenone on sexually immature gilts

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The purpose of this study was to evaluate the effects of a detoxifying agent (DA) added to diets artificially contaminated with zearalenone on sexually immature gilts. Eighty gilts (9.16 ± 0.61 initial body weight (bw) to 23.54 ± 1.4 kg final bw) were used in a randomized complete block design experiment (initial BW as blocking factor) with four treatments, five replicates (blocks), and four animals per experimental unit (pen). Gilts had ad libitum access to diets and water throughout the 28 days feeding experiment. The commercial DA was based on yeast cell wall and activated carbon. The treatments, in a 2x2 factorial design (natural or artificial zearalenone contamination x without or with DA), were: (1) basal diet with ≤ 75 µg/kg natural contamination of zearalenone (negative control); (2) basal diet with ≤ 68 µg/kg natural contamination of zearalenone plus 0.2% of DA; (3) basal diet artificially contaminated with 936 to 968 µg/kg total zearalenone (natural plus added); and (4) basal diet artificially contaminated with 918 to 1,136 µg/kg total zearalenone plus 0.2% DA. The average daily gain (ADG), average daily feed intake (ADFI), feed:gain ratio (F:G), and vulva area was measured. On day 28, twenty animals (5 per treatment) were slaughtered to organ weight (stomach, pancreas, liver, heart, spleen, small intestine, ovary-uterus-vagina-vulva complex) and length (small intestine and ovary-uterus-vagina-vulva complex) measurements. Bile fluid samples were collected for zearalenone and α-zearalenol analysis. The artificially contamination of zearalenone added to diets increased (p<0.001) ovary-uterus-vagina-vulva complex weight (14.79 vs. 37.21 ± 2.62 g) and length (34.57 vs. 43.13 ± 1.09 cm) and vulva area (104 vs. 210 ±10 mm²) compared with natural contaminated diets, while the DA inclusion on artificially contaminated diets was unable to prevent such side effects. The final bw and ADG were lower when artificially zearalenone or DA were independently present in diets (p<0.01) compared to negative control, while when DA was combined with artificially contaminated diet it was able to restore final bw equivalent to negative control. In artificially contaminated diets, higher (p<0.01) levels of zearalenone (843.40 vs. 3.88 ± 77.15 ng/ml) and α-zearalenol (584.97 vs. 2.83 ± 61.78 ng/ml) were detected in the bile fluid than in naturally contaminated diets, while the inclusion of DA in diets decreased such levels to 323.45 and 221.91, respectively. These results suggest a partial efficacy of DA in reduce zearalenone side effects on animals. Factors such as high zearalenone contamination levels, the DA adsorption capacity, and DA levels used on diets might have allowed free residual zearalenone in the animal intestine, available to be absorbed.

Application of fumonisin carboxylesterase FumD as a feed additive (FUMzyme®) counteracts the toxic effect of fumonisin B1 contaminated feed in pigs

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Fumonisin B1 (FB1) is one of the most frequently detected mycotoxins in animal feed. It is known to exert hepatotoxic, nephrotoxic and immunosuppressive effects, and it is the causative agent of the species-specific fatal diseases porcine pulmonary edema in pigs and equine leukoencephalomalacia in horses. The toxic effects of FB1 are due to its inhibition of ceramide synthase, an enzyme involved in sphingolipid metabolism. This inhibition causes a shift in the levels of the sphingolipid metabolism intermediates sphinganine (Sa) and sphingosine (So). Accordingly, the Sa/So ratio is a sensitive biomarker of the effect of FB1. The utilization of enzymes as feed additives for the gastrointestinal degradation of mycotoxins is a promising strategy for the detoxification of feedstuffs. Previously, our group obtained the fumonisin hydrolyzing carboxylesterase FumD from the bacterial soil isolate Sphingopyxis sp. MTA144. The product of this enzymatic conversion, hydrolyzed FB1 (HFB1), shows a greatly reduced toxicity compared to FB1. The commercial application of FumD (FUMzyme®)
represents the first purified mycotoxin degrading enzyme applicable as a feed additive. In this study we investigated the efficacy of FUMzyme® to prevent the toxic effect of FB1 contaminated feed in pigs by facilitating the degradation of the toxin in the gastrointestinal tract. Groups of pigs (n=4) received feed naturally contaminated with fumonisins at different concentrations (3-30 mg/kg), either with or without FUMzyme® amendment (10 U/kg), respectively, for 42 days. To evaluate the gastrointestinal degradation of FB1 we determined the concentrations of FB1 and its degradation products in feces. To assess the toxic effects of the different diets we determined the Sa/So ratio in serum. Addition of FUMzyme® to pig feed facilitated the degradation of FB1 in the digestive tract as evidenced by a significant decrease in the concentration of FB1 and a concomitant significant increase in the concentration of HFB1 in feces. Accordingly, the Sa/So ratio in serum was significantly decreased upon FUMzyme® addition to the diet indicating a significantly reduced toxicity. In conclusion, FUMzyme® was shown to effectively transform FB1 in the gastrointestinal tract of pigs thereby ameliorating the toxic effect of FB1-contaminated feed.

P115: Deoxynivalenol epimerization: an effective route for detoxification with potential agricultural and industrial applications

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Deoxynivalenol (DON) is a type B trichothece mycotoxin that is commonly detected in grains infested with Fusarium species. The presence of this mycotoxin costs the global economy billions of dollars annually in production and trade losses. DON presence in feed is associated with feed refusal and low animal productivity and weight gain. Furthermore, DON is a documented health hazard for humans as it causes both acute and chronic symptoms with changes at the molecular and phosphoproteome levels. Due to the aforementioned factors, the maximum tolerated level of DON in human food chain is restricted to less than 2 mg/kg and to 5 mg/kg in animal feed. Effective methods of controlling, eliminating, and bio-transforming DON are in high pursuit. Our lab reported the isolation of a bacterial strain, 17-2-E-8, identified recently as Devosia mutans that is capable of biotransforming DON aerobically. The efficient transformation is reproducible under wide-range of conditions. The bacterium acts on the C-3 carbon in DON to epimerize the available -OH group and produce 3-epi-DON, eliminating adverse effects during feed consumption. The initial characterization of DON biotransformation indicates it has a high velocity with a fast clearance of DON from the testing medium followed by a gradual accumulation of 3-epi-DON. The observed transformation is enzymatic in nature evident by the inability of inactivated cells and cell-free supernatants to biotransformation DON. Next-generation de novo genome assemblies of multiple Devosia isolates coupled with entire transcriptome mining of D. mutans using RNA-Seq methods were used to highlight clusters of genes that are possibly responsible for the biotransformation function. Further studies are underway to confirm the function of the identified enzymes and optimize them for commercial usage, which will both help the feed/food industry and allow for agricultural and industrial applications to mitigate Fusarium toxin contaminations.

P116: Mycotoxicosis in sows, piglets and pigs

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Mycotoxins are secondary metabolites of fungi from many genera. In this poster, we will focus on two mycotoxins formed by Fusarium molds and their toxicity to swine. Deoxynivalenol (DON) is a trichothecene also known as vomitoxin, causing vomiting, diarrhea and gastroenteritis. Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin. It reduces reproductive performances in sows due to its competition with estradiol in the binding to cytosolic estrogen receptors. In gilts, there are swelling of the vulva, vaginal prolapse and enlargement of the uterus. The absorption of mycotoxins can be controlled at the level of the animal by using mycotoxin eliminating feed additives, preventing their absorption in the gut and allowing them to pass harmlessly through the animal. To evaluate the effect of DON, 2 trials were performed on 80 piglets each. Average daily weight gain and feed conversion ratio was negatively affected at 1.25 and 2.5 mg/kg DON challenge, whereas the addition of 1 kg/t Elitox® could counteract this negative effect. Additionally, different trials on gilts have been performed, contaminating them with 500 μg/kg ZEN. Significant increase of vulva area and relative reproductive tract weight was observed in all trials when gilts received the contaminated diet. Supplementation of 1.5 kg/t Elitox®, could counteract these adverse effects on the reproductive parameters. Ingestion of ZEN resulted in a significant increase in diarrhea incidence, whereas supplementation of Elitox® brought these levels back
to control. Total serum protein and albumin levels were increased due to the presence of ZEN, whereas the addition of Elitox® brought these levels back to control or intermediate values indicating decreased hepatic alterations. T-helper lymphocytes were decreased due to contamination compared to birds supplemented with Elitox®. The amount of circulating cytotoxic T-cells was increased due to ZEN contamination compared with control birds. The number of circulating monocytes numerically increased by the addition of Elitox®.

P117: Evaluation of circulating lymphocytes and gut health in T-2 mycotoxin contaminated broilers

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T-2 toxin is regarded as the most acutely toxic trichothecene mycotoxin (produced by Fusarium species). The toxin is known to inhibit protein synthesis mainly in tissues with a high cell division rate (e.g., liver and intestinal mucosa), inducing cell apoptosis, which may be the cause of severe oral lesions, immunological dysfunctions and impairment in both liver function and intestinal integrity. The objective of this trial is to determine the effect of T-2 toxin on circulating lymphocytes in broilers exposed to contaminated feed. 96 male Cobb® one-day-old broilers were housed in isolators with negative pressure ventilation and split into 3 treatments: a negative control, a positive control containing 800 μg/kg T-2, and a group receiving the contaminated diet (800 μg/kg T-2) combined with a mycotoxin eliminator at 2 kg/t (Elitox®). Early toxicological impacts in immune cells were evaluated through flow cytometry and intestinal impairments were quantified by the amount of goblet cells in the jejunum. T-2 toxin increased the amount of circulating suppressor macrophages, which is an indication of reduced phagocytosis efficiency already after 14 days of exposure. T-helper lymphocytes were also increased due to the action of T-2 toxin, which indicates a higher metabolic cost to sustain homeostasis, as no infective challenge was present and negative control showed significantly less cells. Intestinal integrity was affected by the action of T-2 toxin after 28 days of exposure. Goblet cells were significantly increased in jejunum of intoxicated birds, which may be an indicator of unspecific damage to the intestinal barrier (unspecific inflammation). The use of Elitox® protected animals exposed to the toxin as Elitox® modulated the immune response of such birds to the same level as the negative control.

P118: Determination of blood and immune parameters in broilers exposed to fumonisin

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Fumonisins (FUM) are mycotoxins commonly produced by molds belonging to the Fusarium genus. They are usually identified in poultry feed, because these feed are mainly based on corn. One of the strategies currently available to mitigate the effect of mycotoxins in livestock is the inclusion of anti-mycotoxin additives, with specific adsorbent and/or detoxifying properties against those compounds. The aim of the present trial was to assess the effects of feeding broiler chickens with FUM contaminated feed and its impact on the poultry's immune response and blood variables as well as to evaluate the protective effect of adding an anti-mycotoxin additive to this feed. In total, 96 male Cobb 500 day-old-chicks were divided over 3 isolators of 32 chicks each. Animals were fed one out of the 3 dietary treatments ad libitum until 28 days of age; a corn-soybean meal based control diet, a FUM-contaminated diet (17 mg/kg) or a FUM-contaminated diet supplemented with an antimycotoxin additive. The contaminated diet was formulated by replacing control corn by a naturally contaminated corn with Fusarium mycotoxins. The anti-mycotoxin additive supplemented diet was prepared using 0.2% of a commercial product (Elitox®). Blood samples were taken from 8 animals per treatment at 3, 7 and 14 days of age for evaluating circulating lymphocytes, as well as at 14 and 28 days of age for the determination of plasma proteins, albumin and globulin and serum levels of sphingosine and sphinganine. On day 7, 14 and 28, samples of jejunum were collected from 6 animals per treatment for intestinal mucosa cell measurement. Results showed that FUM had detrimental effects in broilers, resulting in decreased hematocrit and increased albumin, albumin/globulin and Sa:So values in the blood as well as decreased T-helper lymphocyte, activated T-cytotoxic lymphocytes and monocytes concentrations. The addition of the antimycotoxin additive was shown to ameliorate most of the negative effects of FUM.
P119: Effects of the supplementation of yeast cell wall (Safmannann) on the blood serum level of zearalenone, α-zearanol, and β-zearanol in dairy cows fed a zearalenone-contaminated diet

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Zearalenone (ZEN) is a mycotoxin produced by some Fusarium species. When fed to animals, ZEN is transformed to α-zearanol, and β-zearanol which have the same effects or worse than the original compound. When diets of dairy cows are contaminated with this mycotoxin, negative effects in reproduction such as reduced fertility, inflammation of the reproductive tract, and abnormal estrus cycles have been reported in the literature. The objective of this experiment was to evaluate the effect of supplementing a yeast cell wall product (YCW) (Safmannann, Phileo Animal Care, France) on the level of ZEN and its metabolites in the blood and urine of dairy cows fed a diet contaminated with zearalenone. Twenty four Holstein cows were randomly assigned to one of four treatments. The treatments were: (1) control, regular diet containing less than 50 μg/kg of ZEN (CON); (2) positive control, the same diet but containing 1000 μg/kg of ZEN (POS); (3) positive control + 10 g/head/day of YCW (TEN); and (4) positive control + 20 g/head/day (TWE). On day 0 of the experiment, cows were bled to measure the initial levels of ZEN, α-zearanol, and β-zearanol in blood serum because naturally there are small amounts of ZEN present in the grains and silages. Then the diets containing the ZEN were fed for 10 days. On day 10 of the experiment, cows were bled and total urine was collected. Blood and urine were prepared and frozen for future analysis. Samples were analyzed by liquid chromatography, data were transformed to a logarithmic base to obtain a normal distribution and one-way ANOVA analysis was conducted utilizing Statgraphics 5.1. No difference (p>0.05) were observed in the initial values of ZEN, α-zearanol, and β-zearanol in blood serum of cows among groups. After the ten day experimental period, higher (p<0.05) levels of the three compounds were found in the blood serum and urine of the POS group when compared to the other groups. The addition of YCW had numerically lower concentrations of the three compounds evaluated. No difference (p>0.05) between the supplementation of 10 or 20 g/head/day of YCW for any of the parameters measured were found. The supplementation of higher amounts of YCW did not improve the reduction in levels of ZEN and its metabolites in blood serum and urine of dairy cows. This study shows the ability of the YCW to reduce the absorption of the ZEN and its metabolites in dairy cows.

P120: Effects of yeast cell wall on zearalenone toxicosis in prepubertal female gilts

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Zearalenone (ZEN) is a mycotoxin produced by a number of Fusarium species grown on grains, which induce reproductive disorders primarily in pigs. This study was investigated to evaluate the efficacy of yeast cell wall in alleviating ZEN toxicosis in gilts. A total of 40 prepubertal female gilts (Landrace×Yorkshire ×Duroc) with an average body weight of 62.72±1.63 kg were randomly assigned to 4 treatments with 10 replicates of 1 gilt per replicate. The basic diet (C0) was examined using HPLC to ensure mycotoxins level. Treatment 1 (M0) was prepared by replacing 6% of wheat bran in the basic diet with moldy corn core naturally contaminated with ZEA. Treatment 2 (M0 + yeast cell wall) was formulated by adding yeast cell wall to the T1 diet. The final concentration of ZEN in the T1 or T2 diets was 596.86 and 588.24 μg/kg, respectively. Treatment 3 (C0 + yeast cell wall) was composed of C0 adding yeast cell wall, the ZEN content was 85.79 μg/kg. All diets used in the study were isocaloric and isonitrogenous. Nutrient concentrations met or exceeded NRC recommendations (1998). The experiment was lasted for 28 days. Results showed that there had been no negative effects on the growth performance of gilts fed the diet contaminated with 596.86 μg/kg ZEN. Compared with the Control group C0, the vulva size of the prepubertal female gilts in the group M0 increased significantly from days 1 to 28, but the vulva size of gilts in the group M0 + yeast cell wall fed the diet containing yeast cell wall exhibited a significant reduction as compared to vulva size in the group M0, returning to the normal state exhibited by the control group (p<0.05). Piglets fed with 596.86 μg/kg of ZEN (M0) had significantly heavier reproductive organs (p<0.05) than those offered the basic diet (C0). Diets containing 588.24 μg/kg of ZEN supplemented with yeast cell wall (treatment 2) exhibited an obvious reduction in the relative weights of reproductive organs (p<0.05), maintaining them in a normal status showed in the control group. There were no significant changes in the relative weights of other organs (heart, liver, kidney and spleen) among the four groups (p>0.05). This study showed the preventive capacity of the feed additive against ZEN. Positive effects included the prevention of uterine
enlargement and the reduction of the relative weights of reproductive organs. These findings indicate that yeast cell wall can relieve ZEN toxicosis symptoms in gilts.

P121: Aflatoxin contamination and the use of additives alter plasma parameters in ducklings – multiple trial analyses

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Objective of the study was to assess first the effect of aflatoxin contamination (AFLA) and then the impact of antymycotoxins additives on plasmatic contents of ducklings. A first set of data regrouped 6 trials organized in 18 studies (673 Pekin ducklings aged of 1 day; mean duration of 15.4 days). All trials reported side by side comparisons of a mycotoxin-free feed with a diet contaminated with synthetic AFLA (20 to 370 μg/kg). A second set of data regrouped 24 comparisons organized in 15 studies, all reporting side by side comparisons of the use of anti-mycotoxins additives vs. non-contaminated and AFLA diets (2066 Pekin ducklings aged of 1 day; mean duration of 14 days, mean AFLA of 74 μg/kg).

Outcomes selected were plasmatic concentrations in cholesterol, total protein, and albumin. For each set of data, data were analyzed using a mixed model with the TRIAL variable as a random effect. AFLA and treatment variables were considered as fixed effects respectively in the first and second set of data. Mean values were calculated using the LSMEANS procedure of XLstat, weighting the data for the variance among trials. Results showed that AFLA level linearly decreased (p<0.001) total cholesterol (y=1.818 - 0.004x), albumin (y=13.296 - 0.025x) and total protein (y=29.682 - 0.066x) in plasma. This confirmed the validity of this model to assess the intensity of AFLA metabolic effect on animals. Then, among the 17 antymycotoxins additives tested, SRA, a bentonite that had a proven efficient aflatoxin binding ability in vitro (MyT, Pancosma), exhibited the strongest effect on blood parameters of ducklings fed AFLA contaminated diet. The dietary supplementation of SRA counteracted the negative effects of the contamination on cholesterol (+34.0%, p<0.01), albumin (+31.5%, p<0.01), and total protein (+21.9%, p<0.02) and enabled to reach the same plasmatic values as AFLA-free control (p>0.07). These findings confirm the potential of SRA to efficiently limit the negative effects of this mycotoxin contamination on animals.

P122: Degradation of zearalenone using Saccharomyces cerevisiae NCYC R404

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Numerous reports have been published to indicate the global scale of contamination of cereal grains and animal feeds with Fusarium mycotoxins, such as trichotheccenes, zearalenone (ZEN) and fumonisins (D'Mello and Macdonald, 1998; Bhatnagar et al., 1991; Binder et al., 2000). Of these, ZEN is often implicated in reproductive disorders, such as reduced embryonic survival, fetal weights and infertility in livestock production. There are some materials available for adsorption of mycotoxins in animal feed but ZEN can only be partially adsorbed by most of commercially available minerals, e.g., bentonite binders and organic binders, such as yeast cell wall/mannan oligosaccharides (MOS). However, it is known that some micro-organisms are capable either to degrade completely ZEN or transfer it into ZEN metabolites. The aim of the present study was to evaluate ZEN degradation ability of Saccharomyces cerevisiae widely used in animal nutrition for probiotic purposes. The ability of S. cerevisiae to degrade ZEN was evaluated under aerobic and anaerobic conditions. A batch of malt extract broth (MEB) was split into two parts. S. cerevisiae NCYC R404 at 0.1g/l was added to the 1st part of MEB (SCG), whereas the 2nd part of MEB was not supplemented with yeast and served as a control group (CG). The flasks of both groups containing MEB were incubated overnight at 37°C. The next morning, the MEB in both groups was spiked with ZEN for a final concentration of 200 μg/l. The MEB of SCG group was then split again into two parts in order to carry out analysis under aerobic and anaerobic conditions. Anaerobic conditions were maintained by keeping the surface area small and covered with a layer of oil. Samples were taken at every hour up to 8 h and then at 13, 24, 32 and 48 h. The samples were centrifuged and the supernatant was stored in the freezer and the samples were analyzed using LC-MS/MS. Under aerobic and anaerobic conditions, ZEN was degraded by S. cerevisiae R404 to α-zearalenol (α-ZEL) and β- zearalenol (β-ZEL). A faster rate of conversion was determined in the anaerobic environment. Within one hour of reaction more than 90% ZEN was degraded. These anaerobic conditions are close to those found in the rumen and intestinal tract of livestock. The lack of any significant quantity of α-ZEL demonstrates a real reduction in toxicity, since in many circumstances this metabolite has been shown to be 3 times as toxic as ZEN itself whereas the β-metabolite is less toxic than ZEN (Mirocha et al., 2007; Filannino et al., 2011; Frizzell et al., 2011).
The mechanism for the catabolism of the ZEN by yeast has been demonstrated in scientific studies (Mirocha et al., 2007; Filannino et al., 2011) and is attributed to the action of a cytochrome P450 reductase (EC 1.14.-.-) to produce a catechol (Pfeiffer et al., 2009). Subsequent catabolism of catechol follows with the enzyme catechol O-methyltransferase (EC 2.1.1.6) producing a methylated derivative of β-ZEL. Both these enzymes are intracellular and require the cofactors NADPH and S-adenosylmethionine, which can only be found in the intracellular space of the yeast cell. This emphasizes that an uptake mechanism which sequesters the toxin within the cell is the mode of action for degradation of ZEN by *S. cerevisiae* R404. Even if β-ZEL is less toxic than ZEN it still may cause detrimental effects for animal health and productivity. Further research in mycotoxins remediation technologies have shown that both α- and β-ZEL can be further absorbed by various binding materials leading to a complete deactivation of ZEN (unpublished data). To conclude, this work has demonstrated the ability of *S. cerevisiae* R404 to degrade mycotoxin ZEN. The toxin is removed by an uptake mechanism that allows further metabolism to α- and β-ZEL. The initial amount of ZEN was reduced to zero and α-ZEL was not produced in significant quantities clearly indicating a reduction in toxicity.

P123: Enzymatic detoxification of mycotoxins within the bioethanol production process

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The growing U.S. ethanol industry is producing increasing amounts of interesting byproducts for the feed industry, like distiller dried grains with solubles (DDGS). Feeding byproducts of the bioethanol industry to livestock increases the likelihood of exposing animals to higher mycotoxin levels, as mycotoxins present in the raw materials (mainly corn) are not degraded but concentrated approximately threefold during the bioethanol production process. The mycotoxins can cause adverse health effects in the animal, implicating economic losses for the livestock industry. Counter measures for the bioethanol producer so far comprise rejection of specific raw material batches showing high mycotoxin concentrations or blending raw material with high mycotoxin contamination levels with material low in mycotoxins. The objective of this study was to evaluate the application of mycotoxin degrading feed additives for the detoxification of mycotoxins directly within the bioethanol production process. The fumonisin B1 (FB1) degrading enzyme FUMzyme® served as model enzyme in a lab-scale bioethanol process simulation. FUMzyme® was tested in the bioethanol production process from corn naturally contaminated with 4,879 μg/kg FB1 at two concentrations (10 U/kg corn and 100 U/kg corn) compared to contaminated corn without addition of FUMzyme®. Corn slurries with 30% w/w solids were treated with α-amylase at 83°C for 90 min to liquefy the corn starch. Simultaneous saccharification and fermentation was conducted with addition of glucoamylase and a *Saccharomyces cerevisiae* culture at 32°C for 64 h. FUMzyme® was added either before liquefaction (pre-liq) or before fermentation (pre-ferm). Degradation of FB1 and formation of hydrolyzed FB1 (HFB1) were monitored by LC-MS analysis. Both, pre-liq and pre-ferm addition achieved ≥96% FB1 degradation confirmed by HFB1 formation, while no HFB1 was detected in contaminated corn without addition of FUMzyme®. Moreover, FUMzyme® treated mash performed better in terms of mass loss and ethanol concentration and thus ethanol yield compared to the control without antimycotoxin agent. This study presents the first example for the successful application of a mycotoxin degrading enzyme directly within the bioethanol process to produce high quality DDGS with low mycotoxin levels and simultaneously increasing the ethanol yield.

P124: Efficacy of an algoclay-based adsorbent on breeding ducks exposed to polyccontamination of mycotoxins

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*Fusarium* mycotoxins are globally distributed contaminants produced by *Fusarium* species in cereal grains. The adverse effects of these compounds on animal and human health have been reported. The prevention of mycotoxicoses attained by feed additives with mycotoxin adsorbents has also been demonstrated in previous studies. The objectives of the current study were to characterize *Fusarium* mycotoxins in laying ducks with an emphasis on laying rate, egg hatchability and to evaluate the efficacy of an adsorbent containing bentonite-montmorillonite clay interspersed with sulfated polysaccharides extracted from *Ulva* sp., green marine algae (MT.X+). A total of 396, 19-week-old laying ducks (Triet Giang genetic) with 33 birds per replicate and 3 replicates were randomly assigned to each of 4 diets, including (1) control, (2) contaminated, (3) control + 0.1% mycotoxin adsorbent and (4)
contaminated + 0.1% mycotoxin adsorbent for a 13-week period. The experimental parameters analyzed were performance (FCR for kg of feed/10 laid eggs and mortality), egg rate and egg hatchability (after 5 and 17 days of incubation). Feeding diets naturally polycultivated with *Fusarium* mycotoxins containing mainly deoxynivalenol (2.42 µg/g) and fumonisins (1.61 µg/g) slightly increased FCR and mortality while significantly reduced egg rate compared to controls (65.67 vs. 70.98%). The adverse effects of feeding polycultivated diets on egg hatchability were also found after 5 and 17 days of incubation compared to controls (66.67 and 61.33 % vs. 92 and 88.67%, respectively). The tested adsorbent was completely effective in preventing these effects. It was concluded that relatively high concentrations of *Fusarium* mycotoxins can adversely affect egg rate and egg hatchability in laying ducks. The tested adsorbent could be a promising multimycotoxin absorbent for poultry producers.

**P125: Microbial degradation of deoxynivalenol**

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Mycotoxin contamination of food and feed poses major risks for human or animal health and leads to economic losses. Prevention and intervention measures are very well described on the field, but still contaminated batches remain a reality in practice. In order to salvage these resources, feed remediation based on mycotoxin adsorption is already applied through the use of binders. However, adsorption is reversible, pH depending and non-specific. Moreover, these binders negatively influence the transfer of medication to the bloodstream. Therefore, there is need to develop more reliable detoxification strategies. This research focuses on the microbial degradation of mycotoxins, in particularly deoxynivalenol (DON) which frequently occurs in crops in Belgium. Several microbial communities with a possible exposure history to mycotoxins or other complex molecules, are screened for the presence of DON degrading microorganisms. Enrichment cultures of soil and activated sludge showed degradation of DON after two weeks, as analyzed with ELISA, whereas detoxification of DON was confirmed with a bio-assay using *Lemna minor*. Subsequently, several strains, among which one promising isolate related to *Streptomyces* sp. (derived from soil), have been purified and characterization of their degrading capacities is ongoing.

**P126: Use of Actinomycetes for aflatoxins biodegradation**

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*Aspergillus* is a fungal genus contaminating a wide range of food commodities. Aflatoxins are a group of mycotoxins produced by *Aspergillus* and occurring on various commodities such as cereals, nuts, dried fruits and grapes. Many strategies have been developed in the recent years to prevent aflatoxins contaminations at pre-harvest. However, aflatoxins remained frequently found in food products. In Europe, Italy and Spain are already frequently contaminated with aflatoxins. Nevertheless, with global warming, France is being at risk. Thus, the research project AFLAFREE focused on developing biological tools based on bacteria to prevent or/and decontaminate aflatoxins contamination. In this task, we focused on aflatoxins decontamination. A screening of soilborn *Actinomycetes* isolates (including *Streptomyces* spp.) was done. After a 10-days *in vitro* co-incubation with *Aspergillus flavus*, aflatoxins B1 (AFB1) and B2 analysis (HPLC) revealed that these isolates reduced up to 95.6% of the aflatoxins concentration. Among those isolates, we tested their capacity to reduce pure AFB1 in supplemented ISP2 medium. After 4 days at 28°C, some strains were able to reduce AFB1 content up to 84.6%. To better understand the mechanisms involved, we realized adsorption tests in liquid medium on the most AFB1 reducing strains. The results showed no adsorption after 1 h at 28°C. These strains were tested for degradation tests in liquid medium for 4 days. The degradation tests were done for the supernatant and the intracellular extract. The degradation mechanism was find in the supernatant with an AFB1-degradation up to 80% depending on the strain. The metabolites involved are currently under characterization/purification. This study is the first showing AFB1 degradation efficacy among the *Streptomyces* genus. **Acknowledgements.** ANR Aflafree 2011-2015 No.11 003 01, Agrimip Sud Ouest Innovation.
P127: Using enzymes and microorganisms to modify the mycotoxin deoxynivalenol

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Deoxynivalenol (DON) is a trichothecene mycotoxin produced by the fungus Fusarium graminearum that contaminates staple crops such as wheat, barley, and corn when they are infected with this fungus. New strategies are needed to mitigate DON. We screened for microbes that could grow in the presence of 100 mg/kg DON, and found two mixed cultures and two pure cultures that consistently detoxified DON in laboratory experiments. Sequencing analysis of the pure cultures indicated that they were Pseudomonas and Achromobacter. Nuclear magnetic resonance analysis of one of the culture byproducts indicated that DON was converted to 3-keto-DON. In a second approach, we engineered yeast strains to be sensitive to 100 mg/kg DON, and used them to screen library fragments generated from the mixed cultures and the Pseudomonas species and cDNA enzyme sequences created by Integrated DNA Technologies. Three library fragments and two cDNA enzyme sequences were identified that allowed the yeast to grow in the presence of 100 mg/kg DON. In future studies, microbes and enzymes that demonstrated DON detoxification will be tested on contaminated wheat and barley samples. Our research offers a unique approach to reduce DON in these grains, particularly in the context of ethanol co-products.

MISCELLANEOUS TOPICS
P128 – P132

P128: Metabolomics – what does grain chemistry tell us about aflatoxigenic Aspergillus flavus?

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Metabolomic profiling provides insight into the physiological and biological processes of a system under investigation. Information of the metabolomic changes that occurs in corn (Zea mays) during Aspergillus flavus contamination is sparse. In a study to investigate fungal dose-induced metabolite changes in corn, metabolomic profiling was conducted. Conidia from aflatoxigenic A. flavus at different fungal doses classified as control, low, medium and high doses were used. In a 4x3 factorial design, corn kernels were infected with these doses at three stages of crop maturity (R3-milk, R4-dough and R5-dent). Polar and non-polar fractions were subjected to metabolomics profiling using single quadrupole gas chromatography–mass spectrometry (GC-MS). A total of 141 polar analytes and 114 non-polar analytes were detected. Chemometric analyses conducted identified analytes including sugars, fatty acids and amino acids with variations in trends within these analyte groups. Chemometric techniques involved stepwise discriminant, linear discriminant and principal component analyses. Detected biochemical molecules are associated with physiological and biochemical processes that occur during the infection of Zea mays by aflatoxigenic A. flavus. Some of the identified compounds are involved in the glycolytic and tricarboxylic acid pathways. Targeted analyses of selected metabolites are being investigated in the next stage of the experiment. Acknowledgements. The authors are grateful to Queensland Alliance for Agriculture and Food Innovation for Travel Award given to the presenting author. The authors are also grateful to the Federal governments of Nigeria and Australia for the nomination and sponsorship of the PhD scholarship for Titilayo Falade.

P129: Risk ranking of chemical hazards in spices and herbs

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Spices and herbs bring flavor and nutrients to cuisine; however, they may expose consumers to various contaminants, particularly mycotoxins, that pose a risk to human health. Purpose: To rank the risks of
chemical hazards in selected spices and herbs that have the potential for contamination as input for setting up monitoring programs. Particularly, the impact of mycotoxins relative to other chemicals was assessed. A risk ranking toolbox for food and feed related issues (van der Fels-Klerx et al., 2015) was used to systematically select a ranking method. The method was applied to rank various chemical hazards in paprika/chili, black pepper, nutmeg, basil, thyme, and parsley leaves. The severity and probability of the hazards were scored as low, medium, high, or severe. Literature and data were collected from scientific publications, alerting and (national) monitoring data, and other relevant European Union reports and databases to determine the scores. A risk matrix approach was selected to rank the various chemical hazards including natural contaminants such as mycotoxins and plant toxins, agrochemicals, environmental contaminants, such as dioxins, heavy metals, and polycyclic aromatic hydrocarbons, and deliberate contaminants such as dyes. The risk ranking showed that the mycotoxins aflatoxins and ochratoxin A were the riskiest with respect to human health, as well as the pesticides chlorpyrifos and triazophos, and the dye Sudan I. Fumonisins were classified as medium risk compounds and sterigmatocystin as a low risk compound in the selected spices and herbs. A risk matrix provides a transparent risk ranking approach based on available data without the intensive demands required by risk assessments. These results can assist European Union initiatives focusing on sampling strategies for monitoring programs with respect to chemical contaminations in spices and herbs. In addition to mycotoxins, the focus should be on pesticides and the dye Sudan I. Acknowledgements. This research was executed in the framework of the EU-project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the European Union. Financial contributions from the Dutch Ministry of Economic Affairs are acknowledged.

P130: Xenobiotic remediation in niche domination of soil-borne mycotoxigenic fungi – the deletion analysis of fungal lactamases in Fusarium verticillioides

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Beta-lactamase enzymes are well studied because of their huge impact on medicine. Their prominent role is in resistance to beta-lactam (four membered lactam ring) antibiotics including the first and most famous fungus derived medically important antibiotic, penicillin. These antibiotics primarily function by interfering with bacterial cell wall construction. Fungi also have genes that encode canonical beta-lactamase domains. Very little is known about the function of these enzymes in fungi. Clearly, they likely do not act to protect the fungus from beta-lactams since fungi have completely different walls and tend to be unaffected by these drugs. Fusarium species tend to possess large families of beta-lactamase encoding genes. In our fungus of interest, the fumonisin producer Fusarium verticillioides, there are 46 beta-lactamase genes. In fungi, reports describe the functions of only two beta-lactamase genes, one functioning in the synthesis of a secondary metabolite and the other involved in breakdown of a plant xenobiotic. In the case of biosynthesis it was found that it was not the lactamase portion of the protein that is involved in the biosynthesis but rather a second functional domain. In the second case, F. verticillioides was shown to degrade gamma-lactam corn phytoanticipins. Earlier we showed that one enzyme, encoded by FDB1-ORF5 (FVEG_08291), was responsible for detoxification of the corn benzoxazinone phytoanticipins, including BOA. BOA is a gamma-lactam, with a five membered lactam ring, and is cleaved by this enzyme. This result suggests that fungal lactamases have specificity beyond beta-lactams. We are deep into a family-wide gene deletion project to determine the function of these enzymes, with the hypothesis that they are involved in chemical warfare with antagonistic organisms and that their expansion in the genus Fusarium is a driving force for the ubiquitous nature of F. verticillioides in agricultural soils. This presentation will give an update on our results using undergraduates to generate the deletion set by ATMT with our OSCAR methodology.

P131: Microorganisms associated with poor baking quality of wheat

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The proportion of Norwegian wheat used for food has recently been dramatically lower due to both reduced production and poor quality. Furthermore, the Norwegian milling and baking industries have experienced major challenges in utilizing Norwegian wheat due to the instability of factors, such as protein content and gluten functionality, that are of major importance for baking quality. The variation in
the wheat quality can itself cause economic losses for the milling and baking industry due to uncertainty in the marketplace. In the same period as a large variation in baking quality was reported in Norwegian wheat, serious contamination of *Fusarium* spp. and mycotoxins were observed in some grain lots. We have revealed the severe degradation of gluten proteins in some Norwegian wheat samples leading to an almost complete loss in the gluten functionality. The degradation of the gluten appears to be caused by exogenous proteases, and was associated with the presence of *Fusarium* spp., and their metabolites, and other microorganisms in the wheat. Increased knowledge is needed to establish the cause of the poor gluten functionality and to develop control measures to reduce the amount of poor quality wheat entering the food value chain. In 2014, a new project was established to generate deeper knowledge in the interface between gluten functionality and effects of *Fusarium* spp. and other microorganism on wheat quality, and to better utilize Norwegian wheat grown in this challenging environment. A metagenomic analysis, designed to identify microorganisms associated with reduced baking quality, has been undertaken. To study the influence of the identified microorganisms and their metabolites on gluten functionality, wheat plants were inoculated with microorganisms, selected based upon the results of the metagenomics study. *Fusarium* species are among those microorganisms being tested.

P132: MyToolBox – Safe food and feed through an integrated ToolBox for mycotoxin management


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The MyToolBox consortium led by Rudolf Krska (BOKU/IFA-Tulln) will not only pursue a field-to-fork approach along the food and feed chain, but will also consider safe use options of mycotoxin contaminated batches such as microbial energy conversion to efficiently produce biogas and bioethanol assisted by novel enzymes. Intervention technologies considered within MyToolBox include the investigation of genetic resistance to fungal infection, cultural control, the use of novel biopesticides, competitive biocontrol treatment and the development of new forecasting approaches to predict mycotoxin contamination. Research into post-harvest measures includes real-time monitoring during storage, e.g., in China, innovative sorting of crops using vision-technology and novel downstream processing approaches, such as innovative (pre-)milling technology. Research into the effects of baking on mycotoxin levels will provide a better understanding of process factors used in mycotoxin risk assessment. The mycotoxin commodity combinations that will be addressed are the most prevalent *Fusarium* mycotoxins (DON, T-2/HT-2 toxins, ZEN and fumonisins) in wheat, oats, corn and animal feed chains, ochratoxin A in wheat and aflatoxins in corn, peanuts and dried fruit (figs). The developed measures will be combined with existing knowledge and will become accessible via a dynamic web-based MyToolBox e-platform. MyToolBox mobilizes a comprehensive multi-actor approach with 23 partners with > 40% industry participation, including 5 end users from the farming community, agronomists and professionals working in agriculture and food manufacturing.