



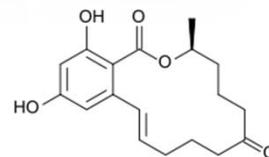
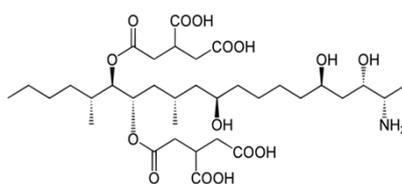
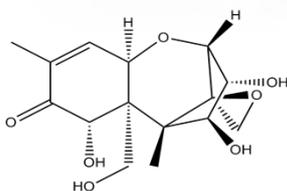
Gesellschaft für Mykotoxinforschung e. V.
Society for Mycotoxin Research



36th Mycotoxin Workshop

16 – 18 June, 2014
Göttingen – Germany

Conference Abstracts



GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

36th Mycotoxin Workshop

Conference Abstracts

Göttingen, Germany

16-18 June, 2014

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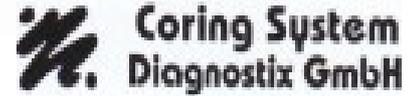
Bianka Gürntke

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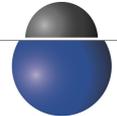
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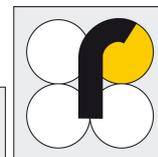


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Program

Monday, June 16th 2014

8:00 Registration

9:00 Welcome

Prof. Dr. Dr. habil. Manfred Gareis - President of the Society for Mycotoxin Research
Prof. Dr. Petr Karlovsky – Chair of the Organizing Committee

Detection and quantification of mycotoxins

Chair: Sarah De Saeger and Karsten Meyer

9:30 Performance and merits of LC-MS/MS based multi-mycotoxin analysis

L01 Michael Sulyok, Rudolf Krska

9:45 Improving measurement certainty of mycotoxin screening methods

L02 Simone Staiger, Mareike Reichel, Scarlett Biselli

10:00 Development of stable isotope dilution LC-MS/MS assays for selected *Alternaria* toxins using biosynthesized labeled internal standards and indications for the paths of generation by fungi

L03 Yang Liu, Michael Rychlik

10:15 Nutrition-related determination of human exposure to mycotoxins in Germany using an improved LC-MS/MS urinary multi-biomarker approach

L04 Johannes Gerding, Tanja Welsch, Benedikt Cramer, Hans-Ulrich Humpf

10:30 Coffee break / Exhibition

Chemistry and biosynthesis of mycotoxins

Chair: Gerhard Adam and Benedict Cramer

11:15 *Cis-Zearalenone*: Instrumental analysis, metabolism and toxicity

L05 Sarah Drzymala, Antje Herrmann, Jennifer Binder, Juliane Riedel, Robert Köppen, Ronald Maul, Leif-Alexander Garbe, Matthias Koch

11:30 North American isolates of *Fusarium graminearum* produce a novel type A trichothecene

L06 Gerlinde Wiesenberger, Elisabeth Varga, Christian Hametner, Romana Stueckler, H. Corby Kistler, Todd J. Ward, Denise Schoefbeck, Rainer Schuhmacher, Franz Berthiller, Gerhard Adam

11:45 PATL, a Cys₆ zinc finger transcription factor is involved in the regulation of the patulin biosynthesis in *Penicillium expansum*

L07 Selma P. Snini, Joanna Tannous, Pauline Heuillard, Enric Zehraoui, Christian Barreau, Isabelle P. Oswald, Olivier Puel

12:00 Apicidin F: Characterization and genetic manipulation of a new secondary metabolite gene cluster in *Fusarium fujikuroi*
L08 Slavica Janevska, Eva-Maria Niehaus, Katharina W. von Bargaen, Christian M. K. Sieber, Henning Harrer, Hans-Ulrich Humpf, Bettina Tudzynski

12:15 Lunch / Exhibition

13:00 Poster session

13:45 Regulation of fumonisin biosynthesis in the rice pathogen *Fusarium fujikuroi*
L09 Sarah M. Rösler, Bettina Tudzynski, Hans-Ulrich Humpf

14:00 An unifying four-enzyme cascade for converting glutathione conjugates into transannular disulfide bridges in fungal ETP toxins
L10 Pranatchareeya Chankhamjon, Daniel H. Scharf, Kirstin Scherlach, Thorsten Heinekamp, Karsten Willing, Axel A. Brakhage, Christian Hertweck

Biological functions of mycotoxins

Chair: Gerlinde Wiesenberger and Rolf Geisen

14:15 A role for carboxylesterases in the chemotype shift in *F. graminearum* populations?
L11 Clemens Schmeitzl, Elisabeth Varga, Alexandra Malachova, Franz Berthiller, Mehrdad Shams, Gerlinde Wiesenberger, Marc Lemmens, Hermann Bürstmayr, Gerhard Adam

14:30 A shift between citrinin and ochratoxin A biosynthesis in *P. verrucosum* supports adaptation to completely different environments
L12 Dominic A. Stoll, Markus Schmidt-Heydt, Rolf Geisen

14:45 Insights into ecological interactions of the meal beetle *Tenebrio molitor* with diverse *Fusarium* species on wheat kernels
L13 Zhiqing Guo, Katharina Döll, Raana Dastjerdi, Petr Karlovsky, Heinz-Wilhelm Dehne, Boran Altincicek

15:00 Coffee break

16:00 Meeting of the Society for Mycotoxin Research

19:00 Welcome reception in the old townhall with the Mayor
Meeting point: Goose Girl Fountain in front of the old townhall (bus stop: "Kornmarkt")

19:30 Dinner at the restaurant Bullerjahn (in the basement of the old townhall)

Tuesday, June 17th 2014

08:00 Registration

Toxicology of mycotoxins, impact on animal and human health (I)

Chair: Siska Croubels and Erwin Märtlbauer

09:00 **Biomarkers as accurate tool for the assessment of mycotoxin exposure at individual levels in Belgium**

L14

Ellen Heyndrickx, Isabelle Sioen, Bart Huybrechts, Mia Bellemans, Mieke De Maeyer, Alfons Callebaut, Stefaan De Henauw, Sarah De Saeger

09:15 **Combined toxic effect of zearalenone (ZEA) and ochratoxin (OTA) on human kidney and mammary cells - protective role of glutathione (GSH) and selenomethionine (SE)**

L15

Virginie Faucet-Marquis, Kheira Hadjeba-Medjdoub, Marie-Carmen Monje, Annie Pfohl-Leszkowicz

09:30 **Ergotism in a rabbit colony?**

L16

Madeleine Gross, Anne Karin Korn, Nina Thom, Stefanie Marschik, Georg Erhardt, Ewald Usleber

09:45 **Unravelling the disposition of the *Alternaria* mycotoxin tenuazonic acid: a toxicokinetic study in pigs and broiler chickens**

L17

Sophie Fraeyman, Mathias Devreese, Nathan Broekaert, Thomas De Mil, Siegrid De Baere, Patrick De Backer, Michael Rychlik, Siska Croubels

10:00 **Effects of deoxynivalenol on expression of immune genes in the intestine of broilers**

L18

Khaled Ghareeb, Wageha Awad, Chimdtseren Soodoi, Soleman Sasgary, Alois Strasser, Josef Boehm

10:15 Coffee break / Exhibition

Toxicology of mycotoxins, impact on animal and human health (II)

Chair: Annie Pfohl-Leszkowicz and Gisela H. Degen

11:00 **MicroRNA-dependent collagen formation induced by ochratoxin A**

L19

Isabell Hennemeier, Michael Gekle, Hans-Ulrich Humpf, Gerald Schwerdt

11:15 **Carry-over of *Fusarium* toxins from naturally contaminated maize to bovine urine**

L20

Janine Winkler, Susanne Kersten, Hana Valenta, Liane Hüther, Ulrich Meyer, Ulrich Engelhardt, Sven Dänicke

11:30 **Citrinin biomarker analysis in urines from Bangladesh**

L21

Nurshad Ali, Khaled Hossain, Meinolf Blaszkewicz, Gisela H. Degen

11:45 **Microbial catabolism of fumonisins: genes, pathways, and applications**

L22

Wulf-Dieter Moll, Verena Klingenbrunner, Markus Aleschko, Doris Hartinger, Elisavet Vekiru, Heidi Schwartz-Zimmermann, Gerd Schatzmayr

12:00 **Is it safe to focus exclusively on non-conjugated toxins? Toxicokinetics, bioavailability and in vivo hydrolysis of 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in chickens and pigs**
L23
Nathan Broekaert, Mathias Devreese, Thomas De Mil, Sophie Fraeyman, Sarah De Saeger, Siska Croubels

12:15 **Lunch / Exhibition**

13:00 **Poster session**

Prevention of mycotoxin exposure and detoxification (I)

Chair: Josef Böhm and Frantisek Malir

13:45 **The dietary exposure of ochratoxin A of ten population groups in the Czech Republic**
L24
Vladimir Ostry, Frantisek Malir, Marcela Dofkova, Annie Pfohl-Leszkowicz, Jiri Ruprich

14:00 **Plant or microbial biotransformation of DON reduces its intestinal toxicity**
L25
Alix Pierron, Sabria Mimoun, Yannick Lippi, Pascal Gourbeyre, Laurence Liaubet, Ting Zhou, Franz Berthiller, Gerd Schatzmayr, Dieter Moll, Isabelle Oswald

14:15 **Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin contaminated white maize**
L26
Limbikani Matumba, Christof Van Poucke, Emmanuel Njumbe Ediage, Sarah De Saeger

14:30 **Mycotoxin contaminations in grape must and wine samples resulting from automated optical grape-sorting process**
L27
Katharina Hausinger, Carsten Fahl-Hassek, Horst-Stefan Klaffke, Gerd Scholten, Dieter Schrenk

14:45 **Mycotoxin degradation in wheat straw by soil faunal communities under field conditions - An ecosystem service for soil health**
L28
Friederike Wolfarth, Stefan Schrader, Elisabeth Oldenburg, Joachim Brunotte

15:00 **Coffee break**

17:00 **Bus transfer to the country estate Nörten Hardenberg**
Meeting point: Long distance bus station 100 m south of the main bus station, which is next to the train station and in front of the J.F. Blumenbach Institute of Zoology and Anthropology, Berliner Str. 28

17:30 **Guided tours of castle or distillery in Nörten Hardenberg**
Address for people travelling independently:
Keilerschänke Nörten-Hardenberg, Hinterhaus 11 A, 37176 Nörten-Hardenberg

19:00 **Conference dinner in the country estate Hardenberg with the poster prize and the Brigitte Gedek Science Award**

23:00-01:00 **Bus shuttle transfer back to hotels in Göttingen**

Wednesday, June 18th 2014

08:00 Registration

Prevention of mycotoxin exposure and detoxification (II)

Chair: Josef Böhm and Petr Karlovsky

09:00 Evaluation of atoxigenic strains of *Aspergillus flavus* for reduction of aflatoxin
L29 contamination of maize on commercial farms in Texas, USA

T. Isakeit, A. Abrameit, W.M. Arnold, M. Jungman, K.J. Matheney, P. McGuill, G.C. Moore,
J.R. Stapper

09:15 Zearalenone detoxification and evolution of lactonase activity in fungi

L30 Delfina Popiel, Grzegorz Koczyk, Adam Dawidziuk, Karolina Gromadzka, Lidia Błaszczyk,
Jerzy Chelkowski

Occurrence, legal and regulatory aspects

Chair: Christoph Gottschalk and Horst Klaffke

09:30 *Stachybotrys chartarum* chemotype S in dried culinary herbs

L31 Barbara Biermaier, Christoph Gottschalk, Manfred Gareis

09:45 Distribution of *Fusarium* mycotoxins and their masked forms in wheat mill fractions

L32 Christine Schwake-Anduschus, Elisabeth Scieurba, Klaus Muenzing, Matthias Proske,
Matthias Koch, Ronald Maul

10:00 Fate of mycotoxins in the cornmeal processing from caryopsis to pre-cooked
L33 porridge

Silvia Generotti, Martina Cirlini, Michele Suman, Chiara Dall'Asta

10:15 Coffee break / Exhibition

11:00 Molecular quantification and genetic diversity of toxigenic *Fusarium* species in
L34 northern Europe and Asia

Tapani Yli-Mattila, Sari Rämö, Veli Hietaniemi, Taha Hussien, Olga Gavrilova, Tatiana
Gagkaeva

11:15 A comprehensive definition of modified and other forms of mycotoxins including
L35 “masked” mycotoxins

Michael Rychlik, Hans-Ulrich Humpf, Doris Marko, Sven Dänicke, Angela Mally, Franz
Berthiller, Horst Klaffke, Nicole Lorenz

11:30 The approach of the European Union Reference Laboratory to promote reliable
L36 measurements

Joerg Stroka

11:45 Closing session

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Abstracts of Lectures

L1

Performance and merits of LC-MS/MS based multi- mycotoxin analysis

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In the recent years, the LC-MS/MS based multi-analyte approach has been successfully applied by numerous groups involved in mycotoxin analysis. Some of the related methods rely on some sort of sample clean-up (e.g. QuEChERS) or on isotopically labelled internal standards to deal with matrix effects, i.e. the decrease (or more rarely the increase) of the signal intensity of an analyte due to co-eluting matrix constituents. These effects are regarded as the biggest problem in LC-MS(/MS) as they negatively affect the accuracy of the methods.

This presentation aims to discuss the analytical performance of the LC-MS/MS based multi-mycotoxin method that has been developed in our group during the past years and that includes no clean-up. Special emphasis will be put on the results obtained from proficiency testing as many authors have expressed their concern about the limited accuracy of this dilute-and-shoot approach. Based on mycotoxin surveys conducted in Africa and Europe the particular strength of the method – its potential to obtain thorough prevalence data and to reveal unexpected findings (such as aflatoxins in maize from central Europe) - will be highlighted.

L2

Improving measurement certainty of mycotoxin screening methods

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Screening approaches have become indispensable in mycotoxin analysis. Two main purposes can be seen: On-site measurement that has to enable rapid decisions on the conformity of lots and multi-toxin monitoring that provides a comprehensive overview on contaminations. For both, results have to be fast available, easy to get and, first of all, reliable.

Rapid on-site screening has to deliver representative results for large lots. Thereby, sampling remains the most critical step. To improve measurement certainty, an innovative approach using dust samples was established. Dust samples were taken during standard grain handling steps and directly analysed using adapted lateral flow tests. Data models allowed calculating contaminations in the lot from concentrations determined in the dust. Measurement uncertainties could be reduced by up to 65% while reducing the measurement time by up to 80% compared to common praxis procedures, showing fitness-for purpose of the new approach.

As monitoring tool, laboratory screening methods have to cope with rising numbers of regulated and emerging toxins. Thereby, a main challenge is to find the best analytical compromise for fast, cost-efficient, but sensitive analysis of a maximum number of diverse molecules. Two ways to improve multi-toxin screenings were evaluated. First, the natural enhancement of mycotoxins in dust samples was used to qualitatively screen grain lots for >30 mycotoxins by means of common LC-MS/MS methods. Secondly, a sensitive method based on HRMS for screening and quantification of various classes of toxins has been developed in a three step approach. Thereby, higher resolution and mass accuracy improved the simultaneous detection of >50 plant- and mycotoxins in common sample matrices.

Best possible combinations of the sensitive monitoring methods, reliable on-site tests and standard reference methods for verification may help to significantly improve mycotoxin control along the whole food chain.

L3

Development of stable isotope dilution LC–MS/MS assays for selected *Alternaria* toxins using biosynthesized labeled internal standards and indications for the paths of generation by fungi

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Labeled *Alternaria* toxins altertoxins (ATXs), alternariol (AOH), and alternariol methyl ether (AME) were synthesized by *Alternaria alternata* in modified Czapek–Dox media using fully ¹³C labeled glucose. The source and concentration of nitrogen in the medium had a decisive impact on the generation of ATXs and tentoxin (TEN), whereas acetate promoted apparently AOH, AME and TEN. Based on the isotopologic *Alternaria* toxins, a stable isotope dilution LC–MS/MS method was developed and applied for food analysis. For ATX I, II, alterperyleneol, AOH, and AME limits of detection ranged from 0.09 to 0.53 µg/kg. The inter- /intra-day relative standard deviations of the method were below 13% and recoveries ranged between 96 and 109%. Among the various commercial food samples, some organic whole grains showed low contamination with ATX I and alterperyleneol, whereas paprika powder, which was heavily loaded with AOH, AME and TEN, showed higher contamination level of ATX I and alterperyleneol. ATX II and stemphytoxin III were not detectable. Maximal concentrations of ATX I and alterperyleneol were 43 and 58 µg/kg and were detected in sorghum feed samples. These contaminations were significantly higher than those in the food samples analysed.

L4

Nutrition-related determination of human exposure to mycotoxins in Germany using an improved LC-MS/MS urinary multi-biomarker approach

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Risk assessment of human exposure to mycotoxins and the subsequent determination of maximum concentration levels in food is commonly based on the correlation of intake and average mycotoxin contamination of food samples across large populations. The individual exposure of subpopulations and groups of higher susceptibility is potentially underrepresented in such evaluations.

An alternative and promising way to address this issue is to measure the occurrence of mycotoxins and their metabolites in human urine. Pilot studies on the exposure of consumers from Africa and southwestern Europe indicate a correlation of contaminated food and biomarker occurrence in urine. Nevertheless, data concerning the exposure of consumers from central Europe still remains scarce. (Shephard G S, Burger H M et al. (2013) Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. *Food Chem Toxicol* 62:217–225) (Solfrizzo M, Gambacorta L et al. (2014) Assessment of Multi-Mycotoxin Exposure in Southern Italy by Urinary Multi-Biomarker Determination. *Toxins* 6 2:523–538).

We have investigated the exposure of 100 individuals from Germany using a rapid and sensitive LC-MS/MS based multi-biomarker approach. The application of scheduled MRM technology allowed the accurate and simultaneous quantitation of 21 biomarkers. Participants of the study filled out a food frequency questionnaire, monitoring their short and long term nutritional habits. This approach gives access to nutrition-related data on mycotoxin occurrence in human urine in a central European population for the first time.

L5

***Cis*-Zearalenone: Instrumental analysis, metabolism and toxicity**

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Zearalenone (ZEN) is a hyperestrogenic mycotoxin that contaminates cereal crops worldwide. Although often neglected, ZEN can undergo isomerization upon exposure to light, thus shifting its olefinic double bond from naturally occurring *trans* to *cis* configuration. Data on *cis*-ZEN is very limited, possibly as a result of poor chromatographic separation from the *trans*-isomer and a lack of standards. Hence, the aim of our studies was to examine the occurrence, fate and risks associated with *cis*-ZEN.

In a first step, chromatographic conditions were set-up which allowed good separation of the two isomers. Using these parameters both, native and ¹³C-labeled *cis*-ZEN, were synthesized, purified, and used as analytical standards for quantification by means of LC-FL and LC-MS/MS. Although no *cis*-ZEN contamination above LOQ was observed in edible oils from German retail markets, a pilot study using *trans*-ZEN contaminated maize-germ oil exposed to daylight revealed more *cis*- than *trans*-ZEN after 2 weeks (Drzymala S, Riedel J, Köppen R, Garbe L, Koch M (2014) Preparation of ¹³C-labelled *cis*-zearalenone and its application as internal standard in stable isotope dilution analysis. *W Myco J* 7:45-52; Köppen R, Riedel J, Proske M, Drzymala S, Rasenko T, Durmaz V, Weber M, Koch M (2012) Photochemical *trans*-/*cis*-isomerization and quantitation of zearalenone in edible oils. *J Agric Food Chem* 60:11733-11740).

Native *cis*-ZEN was then exploited for phase I metabolism studies in rat and human liver microsomes. Results show that *cis*-ZEN is extensively metabolized to a number of oxidative and reductive metabolites. Selected metabolites were purified and then tested for their estrogenicity in the E-Screen model. Overall, the variety of synthesized *cis*-ZEN standards has proven to be valuable for further studies in order to protect consumers from unexpected risks.

L6

North American isolates of *Fusarium graminearum* produce a novel type A trichothecene

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Deoxynivalenol (DON) is a virulence factor of *Fusarium graminearum* on wheat and most likely on other host plants. A large-scale survey of *F. graminearum* (*sensu strictu*) conducted in the northern United States revealed the existence of strains which - based on molecular markers - belong to the 3-acetyl-DON (3-ADON) chemotype, but do not produce DON or nivalenol or any of their known derivatives. Nevertheless, we found that these strains synthesize the trichothecene precursor trichodiene. A compound specifically occurring in these strains was purified and its structure was elucidated. The novel trichothecene, termed NX-2, is identical to 3-ADON with the exception that it lacks the keto group at C-8, which is a hallmark of the typical type B trichothecenes. During colonisation of wheat the new mycotoxin NX-2 is deacetylated to NX-3, like 3-ADON to DON. The toxicity of NX-3 is very similar to that of DON in plant and animal derived in vitro translation systems. By swapping the TRI1 coding regions between the DON producing strain PH-1 and the NX-2 producing strain WG-9 we were able to show that this variant of Tri1p is responsible for specific oxidation at C-7 of the newly discovered trichothecene. At present it is unclear, how prevalent and toxicologically relevant this new mycotoxin is, which escapes conventional detection techniques, and whether its production provides a selective advantage on certain host genotypes.

This work was supported by the Special Research Program SFB Fusarium (SFB F37) from the Austrian Science Fund (FWF).

L7

PATL, a Cys₆ zinc finger transcription factor is involved in the regulation of the patulin biosynthesis in *Penicillium expansum*

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Patulin is a toxic secondary metabolite produced by several fungal species belonging to *Aspergillus*, *Penicillium* and *Byssosclamyces* genera. It is the most common mycotoxin found in apples and apple-derived products such as juice, cider, puree and food intended for young children. The production of this mycotoxin is associated with a blue mold decay of apples, disease caused by *Penicillium expansum*. Patulin is cytotoxic, genotoxic and has immunosuppressive properties but no studies have demonstrated that patulin could be carcinogenic. As other well-known mycotoxins, patulin is the final product of a string of enzymatic reactions. In filamentous fungi, secondary metabolites biosynthetic pathways are enzymatic cascades where enzymes are activated at the same time and the newly synthesized products are consecutively metabolized by the next enzyme. This phenomenon is due to the organization of the genes encoding for the enzymes involved in the biosynthesis in cluster. These genes are co-activated by a transcription factor often located within the cluster. Recently, the cluster of the genes (*PatA-PatO*) involved in patulin biosynthesis was identified in *P. expansum*. A BLAST analysis has revealed that *PatL* encodes for a protein, which presents several characteristic patterns of Cys₆ zinc finger regulatory factor and presents a high homology with AFLR, the specific regulatory factor of aflatoxins biosynthesis. Disruption of *PatL* caused the inability to produce patulin with an important decrease of *Pat* genes expression. In the complemented $\Delta PatL/PatL$ strain, the ability to produce patulin is restored. Pathogenicity studies performed on apples indicated that the $\Delta PatL$ strain infect apples in the same speed as the wild type strain. Based on these results, we can conclude that patulin does not play a role in the development of blue mold decay on apples.

L8

Apicidin F: Characterization and genetic manipulation of a new secondary metabolite gene cluster in *Fusarium fujikuroi*

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The phytopathogenic fungus *Fusarium fujikuroi* is well-studied due to its ability to produce highly bioactive plant hormones, gibberellic acids, that cause the *bakanae* disease of rice plants. In addition, *F. fujikuroi* also synthesizes a vast range of other secondary metabolites (SMs), most of which belong to the SM class of the polyketides. To date, not much is known about non-ribosomal peptide synthetase (NRPS)-derived compounds in this fungus.

We characterized a new NRPS gene cluster responsible for the biosynthesis of the cyclic tetrapeptide apicidin F (APF). APF displays structural similarities to the known histone deacetylase inhibitor apicidin (APS) produced by *Fusarium semitectum*. APF and APS differ in two out of four amino acid constituents while both compounds confer antimalarial activity.

Targeted gene deletion in combination with sensitive, analytical measurements via HPLC, HRMS and NMR revealed insight into the APF biosynthetic pathway. Of particular interest are two biosynthetic mutants that accumulated novel, natural APF analogs with altered amino acid composition, apicidin G and H.

Regarding the regulation of APF biosynthesis, cluster gene expression is induced in the presence of saturating amounts of nitrogen as well as acidic ambient pH and was shown to depend on AreB and PacC, the global nitrogen and pH regulator, respectively. Furthermore, the global regulator Sge1 (controls **SIX** gene expression in *F. oxysporum*) is essential for APF biosynthesis. Overexpression of *APF2*, encoding the atypical, bANK-type (**b**asic region + **a**nkyrin repeats) pathway-specific transcription factor, led to elevated cluster gene expression as well as product formation, even under unfavorable growth conditions. Promoter mutation suggested that Apf2 controls adjacent cluster gene expression via binding to conserved motifs with the consensus sequence “5'-TGACGTCA-3'”.

L9

Regulation of fumonisin biosynthesis in the rice pathogen *Fusarium fujikuroi*

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The maize pathogen *Fusarium verticillioides* is well known for its ability to produce high amounts of the fumonisins of the B series (FB_x). The 17 genes responsible for the biosynthesis of these polyketide mycotoxins are clustered in the fungal genome and are highly conserved among *Fusaria*. Despite the high level of sequence identity of the relevant biosynthesis genes the rice pathogen *Fusarium fujikuroi* produces only very low amounts of fumonisins in shaking culture. The FB_x biosynthesis is repressed by high nitrogen similarly to *F. verticillioides*, but instead of FB₁, like in *F. verticillioides*, FB₂ is the main metabolite in *F. fujikuroi*.

In order to find the reason for low fumonisin production in *F. fujikuroi*, we study the regulatory mechanisms acting on fumonisin biosynthesis in more detail. Addition of different maize ingredients to the medium leads to highly increased FB_x production in *F. verticillioides*. However, only weak induction was observed in *F. fujikuroi*. This effect is probably due to the different host specificity of both fungi.

In order to analyse the pathway-specific regulation the cluster-internal Zn2Cys6 transcription factor Fum21 was overexpressed, leading to a 1000-fold elevated FB_x level even under repressing conditions. Accordingly, the deletion of *fum21* leads to the loss of product formation.

Another focus of our work is the nitrogen regulation of secondary metabolites including fumonisins. Therefore, metabolite levels were measured in deletion mutants of the global nitrogen regulators AreA and AreB. Both GATA transcription factors act as positive regulators of the fumonisin biosynthesis as no FB_x are produced in these mutants. This is the second example for secondary metabolites, besides gibberellins, that are consistently affected by AreA and AreB in *F. fujikuroi*.

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L10

An unifying four-enzyme cascade for converting glutathione conjugates into transannular disulfide bridges in fungal ETP toxins

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Epipolythiodioxopiperazines (ETP) are toxic fungal secondary metabolites that have been implicated in human, animal, and plant diseases. The prototype of this class of microbial toxins is gliotoxin which plays a significant role in mediating the virulence of the human pathogen *Aspergillus fumigatus*. The biological activity of all ETPs critically depends on their structurally intriguing transannular disulfide bridge. This signature residue is capable of inactivating various proteins by thiol conjugation and generates reactive oxygen species by redox cycling. Whereas the biosynthesis of the diketopiperazine core of gliotoxin by a non-ribosomal peptide synthetase was studied some time ago,[1] surprisingly, little was known about the formation of the sulfur bridge. Recently, we unveiled critical steps in enzymatic sulfurization by a specialised glutathione S-transferase and epidithiol formation by a twin carbon–sulfur lyase. However, the exact nature of the S- nucleophile, the first sulfur-bearing intermediate in gliotoxin biosynthesis, and its enzymatic conversion had remained elusive. Here we reveal the structure of this key intermediate and its enzymatic conversion into the bis-cysteine adduct by a glutamyltransferase and a metal-dependent dipeptidase. Moreover, we also present the first successful reconstitution of a four-enzyme cascade to yield an intact ETP in a one-pot reaction [2].

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L11

A role for carboxylesterases in the chemotype shift in *F. graminearum* populations?

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A shift in *F. graminearum* populations, favoring the 3-acetyl-deoxynivalenol (3-ADON) over the 15-acetyl-deoxynivalenol (15-ADON) chemotype, has been observed in many parts of the world (Ward et al. (2008)). An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *F. graminearum* in North America. *FG & B* 45(4):473–484). The order of toxicity is 15-ADON > DON >>> 3-ADON at the ribosome. So it is unclear why maintaining the C3-acetyl group could be of advantage for the invading fungi. Our hypothesis is that the C3-acetyl group prevents detoxification of DON to DON-3-O-glucoside by cytosolic plant UDP-glucosyltransferases. There is evidence that this detoxification mechanism is tightly linked to resistance QTL Fhb1 of wheat (Lemmens et al. (2005) The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. *MPMI* 18(12):1318-1324). Yet, the toxin has to be deacetylated eventually, to be able to interact with the ribosomal target. The deacetylation reactions are not only catalyzed by the *Fusarium* Tri8 protein but also by currently uncharacterized plant carboxylesterases (CXEs). Deacetylation of ADONs was observed in the monocot model system *B. distachyon*, and in crop plants like wheat. We started to characterize this reaction in a double haploid wheat population from a cross between the susceptible variety 'Remus' and the resistant 'CM-82036'. First results suggest that there indeed are differences in deacetylation of ADONs.

Considering the fact that the resistance QTL Fhb1 is now common in North America where the shift in *Fusarium* populations was observed for the first time, plant CXEs located in the endoplasmic reticulum could partly release DON from 3-ADON, thereby assisting the chemotype shift.

L12

A shift between citrinin and ochratoxin A biosynthesis in *P. verrucosum* supports adaptation to completely different environments

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The FAO estimates that up to 25% of the world's crop harvest is contaminated with filamentous fungi or their mycotoxins. For this reason it is important to understand the regulation of mycotoxin biosynthesis in order to develop prevention strategies to reduce the mycotoxin contamination of food and feed. In this study, the regulation of the biosynthesis of ochratoxin A (OTA) and citrinin (CIT), two hepato- and nephrotoxic mycotoxins produced by *P. verrucosum*, were investigated. In *P. verrucosum* the production of OTA and CIT is mutually regulated. On NaCl-rich media the biosynthesis of CIT is reduced, whereas that of OTA is increased. It could be shown that the production and excretion of the chloride-containing OTA molecule ensures cellular chloride-homeostasis under hypersalinic growth conditions. Changes in the concentration of NaCl in the environment are transmitted to the transcriptional level by the HOG-MAPK signal cascade, which results in an adaptation of gene expression. Ochratoxin biosynthesis seems to be regulated by the Hog-MAPK-pathway. Western Blot experiments showed a correlation between the phosphorylation status of the HOG1-homologue of *P. verrucosum* and induction of OTA biosynthesis. Inactivation of *hog1* in *P. verrucosum* by gene knock out abolishes OTA biosynthesis under high NaCl conditions but not that of CIT. In contrast, under oxidative stress conditions the biosynthesis of OTA is reduced and CIT production is increased instead. Changes in the oxidative status are often transmitted to the transcriptional level by a G-protein/cAMP/PKA signal cascade. In the current analysis a correlation between internal cAMP-levels and biosynthesis of CIT could be demonstrated. External application of cAMP resulted in a reduced biosynthesis, suggesting the involvement of such a signal cascade in the regulation of CIT biosynthesis. Citrinin is described to have antioxidative properties, which makes the induction of CIT biosynthesis under oxidative conditions favorable for the fungus. These results suggest that in *P. verrucosum*, the biosynthesis of OTA or CIT apparently act as an adaptation mechanism to hypersaline respectively oxidative stress conditions.

L13

Insights into ecological interactions of the meal beetle *Tenebrio molitor* with diverse *Fusarium* species on wheat kernels

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Species of the fungal genus *Fusarium* have significant agro-economical and human health-related impact by infecting diverse crop plants and synthesizing diverse mycotoxins. Here, we investigated interactions of grain-feeding *Tenebrio molitor* beetle larvae with four grain-colonizing *Fusarium* species on wheat kernels. Since numerous metabolites produced by *Fusarium* spp. are toxic to insects, we tested the hypothesis that the insect senses and avoids *Fusarium*-colonized grains. We found that only kernels colonized with *F. avenaceum* or *Beauveria bassiana* (an insect-pathogenic fungal control) were avoided by the larvae as expected. Kernels colonized with *F. proliferatum*, *F. poae* or *F. culmorum* attracted *T. molitor* larvae significantly more than control kernels. The avoidance and preference behaviors correlated with larval feeding as well as larval weight gain. Interestingly, *T. molitor* larvae that had consumed *F. proliferatum*- or *F. poae*-colonized kernels had similar survival rates as groups fed with control kernels. Larvae fed on *F. culmorum*-, *F. avenaceum*- or *B. bassiana*-colonized kernels had elevated mortality rates. Chemical analyses confirmed the production of the following mycotoxins by the fungal strains on the kernels: fumonisins, enniatins and beauvericin by *F. proliferatum*, enniatins and beauvericin by *F. poae*, enniatins by *F. avenaceum*, and deoxynivalenol and zearalenone by *F. culmorum*. Most of these mycotoxins were also detected in larvae that fed on respective kernels. Our results indicate that *T. molitor* larvae have the ability to sense potential survival threats of kernels colonized with *F. avenaceum* or *B. bassiana*, but not with *F. culmorum*. Volatile compounds potentially along with gustatory cues produced by these fungi may represent survival threat signals for the larvae resulting in their avoidance. Interestingly, although *F. proliferatum* or *F. poae* produced fumonisins, enniatins and beauvericin during kernel colonization, the larvae were able to use those kernels as diet without exhibiting increased mortality rates. Consumption of *F. avenaceum*-colonized kernels, however, resulted in higher larval mortality; these kernels had significantly higher enniatin levels than *F. proliferatum* or *F. poae*-colonized ones suggesting that *T. molitor* have the evolved capability of tolerating or metabolizing those toxins to some content.

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L14

Biomarkers as accurate tool for the assessment of mycotoxin exposure at individual levels in Belgium

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Human exposure assessment to mycotoxins is often based on calculations combining occurrence data in food with population data on food consumption. Because of limitations inherent to that approach, biomarkers have been proposed as a suitable alternative. Individual variation in absorption, distribution, metabolism and excretion is integrated into the formation of the biomarker, whereby a more accurate assessment of exposure at individual level can be performed.

The BIOMYCO study is designed to assess mycotoxin exposure using urinary biomarkers. Over different seasons of 2013 and 2014, morning urine was gathered in a representative part of the Belgian population according to a designed study protocol, whereby 140 children and 278 adults were selected. Every participant completed a food frequency questionnaire to assess the consumption of relevant foodstuffs of both the day before the urine collection and the previous month. Validated LC-MS/MS methods were used to analyse aflatoxins, fumonisins, ochratoxin A, trichothecenes, zearalenone and their metabolites in morning urine. The protocol was approved by the ethical committee of the Ghent University Hospital.

The BIOMYCO study is the first study whereby a multi-toxin approach is applied for mycotoxin exposure assessment in adults and children on a large-scale. Moreover, it is the first study that describes the exposure to an elaborated set of mycotoxins in the Belgian population. Besides providing descriptive data on mycotoxin exposure, exposure of different subgroups (age, gender, region) were compared. Furthermore, correlations between urinary mycotoxin concentrations and food consumption were made. In more than 60% of the urine samples analysed in the first seasons, deoxynivalenol and its main metabolites, 3- and 15-glucuronide were found. Also citrinin and ochratoxin A were detected in more than 50% of the samples. Study design, methods and results of the whole sampling period will be discussed.

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L15

Combined toxic effect of zearalenone (ZEA) and ochratoxin (OTA) on human kidney and mammary cells - protective role of glutathione (GSH) and selenomethionine (SE)

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OTA, a nephrotoxic mycotoxin produced by several *Penicillia* and *Aspergilli*, contaminates several foodstuffs. It is implicated in Balkan endemic nephropathy and urinary tract tumours. ZEA, an estrogenic mycotoxin produced by several fusaria, is often present simultaneously with OTA. The aims of this study were to obtain information about the synergistic effect of ZEA and OTA on human kidney cells (HK2) and mammary cells (MCF-7). Cells were treated by increasing amount of ZEA, OTA or both of them ranging from 10 nM to 100 µM. The cell viability was checked by MTS test. The genotoxicity was evaluated by detection of DNA adduct. The metabolic pathway was analyzed by HPLC. The cell viability is differently affected depending of the mycotoxins and the range of exposure. A non-monotone dose response was observed when MCF7 were exposed to OTA or ZEA individually or in mixture, indicating an endocrine disrupting effect. In contrast, OTA decrease of HK2 cell viability is exemplified by ZEA. Interestingly, exposure to low concentrations to both toxins simultaneously induces toxic effect even though individually the mycotoxins did not induce any effect. OTA and ZEA induce DNA adduct in both cell lines. Simultaneous exposure leads to increase of OTA specific DNA adduct, notably C8dG OTA. GSH significantly reduces DNA adduct both in kidney and mammary cells whatever the conditions of exposure (i.e. mycotoxin individually or in mixture). SE reduces significantly DNA adduct only in MCF7, whereas it increases genotoxicity of both mycotoxins in HK2. Biotransformation of OTA into OTHQ (ochratoxin quinone derivative) was linked to genotoxicity. Simultaneous presence of ZEA shifts the metabolic pathway of OTA. Human kidney cells were more sensitive to OTA whereas the mammary cells were more sensitive to ZEA. The data indicate clearly that exposure to low concentration of mycotoxins which is considered as safe when they are present together can lead to dramatic effect.

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L16

Ergotism in a rabbit colony?

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During a period of eight weeks, 15 juvenile rabbits, in a colony of 103 animals, spontaneously showed tail lesions and necrosis. Similar symptoms including gangrene and terminal necrosis of the extremities is associated with ergotism in several species. Although little is known with regard to rabbit ergotism, some published data suggests that *Leporidae* are highly sensitive towards ergot alkaloids. Other causes (viral, bacterial, malnutrition, automutilation) were not found, so we suspected an acute mycotoxicosis by ergot alkaloids. Pelleted feed and fecal samples of rabbits were analysed for mycotoxins by using enzyme immunoassays. Feed samples were collected from 20 kg bags of complete diet used for this colony (44 bags, 3 different lots) and analysed for total ergot alkaloids and ergotamine. Four samples from one batch were each analysed for fumonisins, deoxynivalenol, T-2/HT-2 toxin, and zearalenone. Ergot alkaloid levels in pooled fecal samples of affected animals were compared with that of clinically symptom-free animal groups from other husbandries. Ergot alkaloids were detected in all 44 feed samples (140 – 1700 ng/g, mean 410 ng/g), ergotamine was the dominant compound (140 - 910 ng/g, mean: 370 ng/g). In control feed samples, ergot alkaloids were much lower. Low levels of fumonisins (5.29 - 6.46 ng/g), deoxynivalenol (260 - 539 ng/g), T-2 toxin/HT-2 toxin (8.72 - 16.9 ng/g), and zearalenone (105 - 132 ng/g) were also detected. Feces samples of the animals with clinical symptoms were highly positive for total ergot alkaloids in a concentration range of 51 – 181 ng/g (mean 110 ng/g, n = 20) and ergotamine 43.8 – 259 ng/g (mean 140 ng/g, n = 22), feces of symptom-free animals were essentially negative for ergots (20 ng/kg). In conclusion, the results suggest that ergot alkaloids may have played a role in disease causation in the rabbit colony under study, but no clear evidence could be obtained. Since professional rabbit farming increasingly uses cereal-based diets, the risk from ergot alkaloid contamination would justify further studies, e.g. to clarify the tolerable levels for ergot alkaloids in rabbits.

L17

Unravelling the disposition of the *Alternaria* mycotoxin tenuazonic acid: a toxicokinetic study in pigs and broiler chickens

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Alternaria fungi can contaminate several crops in the field, but also during refrigerated transport and storage. *Alternaria* species can produce a variety of mycotoxins, with the most prevalent ones being alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA). (1)

Streit et al. (2013) analyzed more than 80 feed and feed raw material samples on the presence of mycotoxins. TeA was present in 65% of the feed samples. (2) Despite the frequent contamination of food and feed with these toxins, little is known about their *in vivo* toxic effects. For a better understanding of their potential hazardous effects in animals, knowledge on their toxicokinetic properties is essential. Therefore, the aim of this study was to determine the toxicokinetics and absolute oral bioavailability of TeA in both pigs and broiler chickens.

A toxicokinetic study was performed in pigs and broiler chickens (n=8). Each animal received 0.05 mg TeA/kg body weight via a single stomach (pig) or crop bolus (chicken) and via a single intravenous injection in a two-way cross-over design. The main toxicokinetic characteristics were calculated (WinNonlin, Pharsight, USA), using one-compartmental modelling. Additionally, the absolute oral bioavailability was calculated. Plasma concentration-time curves suggest that TeA is well absorbed after oral administration in both pigs and chickens. The final toxicokinetic parameters and absolute oral bioavailability will be presented at the 36th Mycotoxin Workshop.

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L18

Effects of deoxynivalenol on expression of immune genes in the intestine of broilers

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Deoxynivalenol (DON) targets mainly the intestinal tract which is the first barrier against ingested feed contaminants and luminal microbes. The intestines have protective mechanisms such as physical, chemical, and immunological barriers which are significant to maintain homeostasis. Alteration of one of these barriers by DON may lead to diseases. Therefore, it is important to identify the effect of DON on gut epithelium.

In chickens, a significant decrease of mRNA levels of interleukin 1 beta (IL-1 β), transforming growth factor beta receptor I (TGFBR1) and interferon gamma (IFN- γ) was observed following DON ingestion in jejunum; indicating that DON when fed for long period can produce immunosuppression and alter the intestinal immune responses. Furthermore, a commercial antidote for DON Mycofix Select (Biomim GmbH, Herzogenburg, Austria) was found to counteract the impacts of DON on IL-1 β and TGFBR1.

It seems likely that DON provokes its toxicity in the intestines through alternation of genes responsible for immune response. DON may similarly affect the intestines of humans by its immunosuppressive effects and may lead to a major health threat.

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L19

MicroRNA-dependent collagen formation induced by ochratoxin A

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Ochratoxin A (OTA) is an ubiquitous food contaminating mycotoxin suspected to cause fibrotic kidney diseases but the underlying molecular mechanisms, especially the involvement of non-coding RNAs in these processes, are unknown. Therefore, we investigated the role of the anti-fibrotic miR-29b in OTA-induced alterations of cellular collagen homeostasis in human renal cells. Our results demonstrate that exposure of human embryonic kidney cells (HEK293) to nanomolar OTA concentrations led to an increase of collagen I, III and IV protein amounts without changes in collagen mRNA expression level, indicating post-transcriptionally mediated mechanisms probably including microRNAs and 3' untranslated regions (3'UTR) of collagen mRNAs. The involvement of 3'UTRs was confirmed by enhanced luciferase activity of a collagen1A1-3'UTR reporter plasmid after OTA exposure. As collagen1A1-3'UTR contains amongst others seed regions for miR-29b, we concentrated on miR29b which is described to possess anti-fibrotic properties. OTA enhanced the luciferase activity of a reporter plasmid containing the seed region of miR-29b showing that OTA diminishes miR-29b action. This could be explained by an OTA-induced altered intracellular distribution of miR-29b leading to decreased cytoplasmic abundance of miR-29b. The dependence of the OTA-mediated fibrotic effects on miR-29b function was further underlined by the observation that miR-29b added in high amounts (miR-29b clamp) completely prevented OTA-induced collagen formation.

In summary, we show that OTA has the potential to initiate or support the development of fibrotic kidney diseases by involving post-transcriptional regulatory mechanisms carried by at least miR-29b. OTA reduces the anti-fibrotic impact of miR-29b and thus enhances collagen protein expression. These findings allow a new perspective on how the exposure to nanomolar OTA concentrations can lead to fibrotic tissue alterations.

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L20

Carry-over of *Fusarium* toxins from naturally contaminated maize to bovine urine

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Mycotoxins like zearalenone (ZEN) and deoxynivalenol (DON) and their degradation products can potentially be used as biomarkers in urine to assess the exposure of humans and animals. Therefore, a dose-response study with dairy cows, which were fed rations with increasing concentrations of ZEN and DON contaminated maize was carried out to examine the ZEN and DON concentration in urine.

Three groups of dairy cows (n=10 in each) were fed over 13 weeks starting one week after calving with following toxin concentrations: Control (CON, 0.02 mg ZEN and 0.07 mg DON per kg dry matter (DM)), *Fusarium*-50 (FUS, 0.33 mg ZEN and 2.62 mg DON per kg DM), FUS-100 (0.66 mg ZEN and 5.24 mg DON per kg DM). Urine samples were collected one day before the feeding trial started, after one week, at week nine and at the end of the feeding trial from each cow. Urine samples were purified by solid phase extraction and ZEN, DON and their metabolites were simultaneously determined by LC-MS/MS analysis. Furthermore, creatinine concentration of each urine sample was analysed by HPLC-DAD. The analysed toxin concentrations were related to those of creatinine to account for variation in the daily amounts of excreted urine.

Besides the parent toxins ZEN and DON, the metabolites α -zearalenol, β -zearalenol and de-epoxy-DON (de-DON) were detected in the urine samples of cows fed the *Fusarium* contaminated maize. Furthermore, traces of zearalanone (up to 1.1 ng/mL), α -zearalanol (up to 1.3 ng/mL) and β -zearalanol (up to 1.4 ng/mL) could be detected in some urine samples. Based on results of the analysis with β -glucuronidase treated and untreated samples conjugated toxins were the major urinary metabolites. Moreover, DON was almost completely metabolized to de-DON (83-98%) independent of the DON exposure. Linear relationships between toxin intake and urine levels could be established.

In conclusion, ZEN and DON and their detected metabolites could be used as biomarkers of exposure.

L21

Citrinin biomarker analysis in urines from Bangladesh

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Background: Citrinin (CIT) is a nephrotoxic mycotoxin contaminant in food commodities, and can co-occur with ochratoxin A (OTA). Presence of OTA in maize from Bangladesh was reported [1], but CIT contamination of foods or feed in Bangladesh has not been studied. It was the aim of this study to analyze CIT and its metabolite dihydrocitrinone (HO-CIT) in urines from two Bangladeshi cohorts, since biomonitoring provides the best approach to assess human exposure to contaminants from various sources and by all routes.

Methods: Urines were collected from inhabitants of a rural (n=32) and an urban (n=37) area during May 2013. A sensitive method [2] was used for clean-up of urine by immunoaffinity column and subsequent LC/MS-MS analysis of the extracts. The LOD for CIT and HO-CIT were 0.02 ng/mL and 0.05 ng/mL urine, respectively.

Results: CIT and HO-CIT were detectable in 94% and 73% of all urine samples. Urinary biomarker levels did not show significant correlations with age, sex and body mass index of the donors. But, excretion of CIT and its metabolite (total) was significantly higher in rural people (mean 1.1 ± 1.9 ng/mL) than in urban people (0.14 ± 0.14 ng/mL), indicative of differences in mycotoxin exposure.

Discussion: Most of the people in the rural cohort of Bangladesh are farmers or farm workers involved in grain production while urban people are office workers or students. Food habits also differ between the rural and the urban cohort. It can be concluded that contaminated food commodities are major contributors for CIT exposure in humans in Bangladesh. The results of biomonitoring will be compared to new data for (n=50) German volunteers.

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L22

Microbial catabolism of fumonisins: genes, pathways, and applications

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Genes for fumonisin biosynthesis have been found in the genomes of fungi from separate orders. The evolutionary relationship between these gene clusters suggests that fumonisins have been present in the environment for a long time. Therefore it seems probable that microbes would have evolved the ability to metabolise fumonisins, and fumonisin catabolism genes and pathways have indeed been found: Degradation of fumonisins by black yeast strains and by an unclassified bacterial strain were previously described in literature and in patent applications, and we discovered a new fumonisin degrading Alphaproteobacterium, *Sphingopyxis macrogoltabida* MTA144. We determined the whole genome sequence of this strain and identified a fumonisin catabolism gene cluster. We characterised the gene cluster by bioinformatic sequence analysis, by cloning and expression of genes for catabolic enzymes, by fumonisin biotransformation assays with strain MTA144 and purified recombinant enzymes, and by purification and structure determination of reaction products. We found that the fumonisin catabolism gene cluster of MTA144 is regulated and inducible, and growth of the strain is enhanced by fumonisins in growth medium. Both known bacterial fumonisin catabolism pathways start with hydrolytic release of tricarballic acid side chains, catalysed by fumonisin esterases, just like in the yeast pathway. The second step of fumonisin catabolism of yeast and bacterial strains yields the same reaction product, 2-keto hydrolysed fumonisins, but the reaction is catalysed by an amino oxidase in yeast strains, and by an aminotransferase in bacterial strains. Further fumonisin catabolism by strain MTA144 involves an alcohol dehydrogenase. Application of enzymes for fumonisin detoxification has been attempted, and fumonisin esterase FumD has been developed as feed enzyme for hydrolysis of fumonisins in the gastrointestinal tract of piglets.

L23

Is it safe to focus exclusively on non-conjugated toxins? Toxicokinetics, bioavailability and *in vivo* hydrolysis of 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in chickens and pigs

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The research towards the toxicity and toxicokinetics of deoxynivalenol (DON) has made significant progress during the last years. As the information and insight on native mycotoxins increases, the focus of this research domain tends to shift towards the gathering of information on mycotoxin conjugates. These conjugated mycotoxins can be produced by either fungal (a.o. acetylation) or plant (a.o. glycosylation) metabolism. Conversion of these conjugated mycotoxins back to their native form by *in vivo* hydrolysis cannot be excluded, implying a risk of underestimation of the degree of contamination upon analysis.

The goal of this study was to determine the toxicokinetic characteristics, absolute oral bioavailability and degree of *in vivo* hydrolysis of 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON) in broiler chickens and pigs. To this purpose firstly an LC-MS/MS method was developed and validated to determine DON, 3ADON and 15ADON, as well as the major *in vivo* metabolite deoxy-deoxynivalenol (DOM-1) in pig and chicken plasma.

Next, two animal trials, one for pigs and one for broilers, in a two-way cross-over (per os (PO) and intravenous(IV)) design were set up. The doses that were administered represented a worst case scenario within the European Union legal framework.

Plasma concentration-time profiles were constructed after LC-MS/MS analysis. The toxicokinetic characteristics were determined by means of WinNonlin software, using non-compartmental modeling to determine the AUC, C_{max} etc... Additionally, the absolute oral bioavailability and degree of *in vivo* hydrolysis were calculated.

Results indicate a low absolute oral bioavailability for all three compounds (10-20%) in broilers. A profound hydrolysis of 3ADON, and to a lesser extent of 15ADON, to DON after PO and IV adminis.

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L24

The dietary exposure of ochratoxin A of ten population groups in the Czech Republic

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Ochratoxin A (OTA) is a nephrotoxic and nephrocarcinogenic mycotoxin. OTA is produced by *Aspergillus* and *Penicillium* species and is a common contaminant of various foods of plant and animal origin (Ostry V, Malir F, Ruprich J (2013) Producers and important dietary sources of ochratoxin A and citrinin. *Toxins* 5:1574-1586). Tolerable Weekly Intake of 120 ng/kg bw/week was derived for OTA based on its nephrotoxicity properties in pig. Based on carcinogenic properties of OTA, the negligible cancer risk intake has been assessed to be 4 ng OTA/kg bw/day in Health Canada (Malir F, Ostry V, Novotna E (2013) Toxicity of the mycotoxin ochratoxin A (OTA) in the light of recent data. *Toxin Rev* 32:19–33). The objective of this study is assessment of dietary exposure and health risk characterization of OTA for ten population groups (age 4-90 years, both sexes). Eighty six kinds of food of vegetable and animal origin were collected in 4 sampling terms at 12 regions of the Czech Republic in the years 2011-2013. Total number of 1032 food samples was collected. Validated and accredited HPLC-FD method was employed for OTA determinations. Limit of quantification of the method varied between 0.01-0.2 ng/g according to the type of sample matrix. In total, maximum amount of dietary exposure for “average consumer“ to OTA was estimated at 3.9 ng/kg bw/day in children 4-6 years old. Important exposure sources for this population group were tea, grain-based products, confectionery, meat and meat products and juice. Mean dietary exposure for “average consumer“ to OTA for the population groups of men and women 18-59 years old was estimated at 1.7 and 1.2 ng/kg bw/day respectively. Beer was the main contributor of OTA dietary exposure in men 18-59 years old. Tea and grain-based products were identified as the main contributors for women 18-59 years old. The maximum amount of dietary exposure to OTA from coffee and wine were identified in population group of women 18-59 years old.

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L25

Plant or microbial biotransformation of DON reduces its intestinal toxicity

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Deoxynivalenol (DON) is a frequent mycotoxin in grains, produced by *Fusarium* fungi, which demonstrates multiple side effects such as modulation of immune responses, reduced feed intake and weight gain or impairment of the intestinal barrier function. Among animal species, pigs are the best model for humans and are very sensitive to DON. In wheat, DON can be conjugated to glucose to form DON-3- β -D-glucoside (D3G). Some bacteria isolated from digestive tracts or soil, are also able to de-epoxydize or epimerize DON to metabolites such as deepoxy-deoxynivalenol (DOM-1) or 3-epi-deoxynivalenol (epi-DON). The toxicity of these DON metabolites is poorly documented. The intestine is the first organ exposed to these molecules and so constitutes a relevant model. The aim of this study was to compare the intestinal toxicity of three DON metabolites (D3G, DOM-1 and epi-DON) with the one of DON. Intestinal explants from 6 pigs were treated with 10mM DON, D3G, DOM-1 or epi-DON for 4 hours and transcriptomic analysis was performed using an "Agilent Porcinet 60K". Among the 34,775 probes expressed in array, 747 were differentially expressed in DON-treated explants when compared to Mock-treated ones. It represents 323 genes. It encompasses genes involved in various processes including inflammatory response, cell death and survival as well as hematological system development and function. Data obtained by microarray analysis were confirmed by real time qPCR, significant increase of the expression of the pro-inflammatory cytokines IL-1beta, IL-8, IL-17, TNF-alpha, IL-1alpha, IL-22, IL-12p40 are observed in DON treated explants. In contrast, no genes were differentially expressed between control and D3G, DOM or 3-epi-DON treated explants as demonstrated by DNA array and qPCR analysis. In conclusion, our data confirm the intestinal toxicity of DON and demonstrate that de-epoxydation, glucosylation or epimerization of DON decrease the toxicity of this mycotoxin in this intestinal model.

L26

Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin contaminated white maize

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Maize is one of the major staple foods of sub-Saharan Africa and is consumed as whole and dehulled grain. In this region, the majority of the population are subsistent consumers who have little or no access to mycotoxin testing of their food. In an attempt to develop feasible dietary mycotoxin exposure reduction strategies for the population a three-factorial design experiment was conducted to examine and compare the efficacy of hand sorting, flotation, dehulling and combinations thereof in removing naturally occurring aflatoxins, fumonisins, nivalenol, deoxynivalenol, zearalenone and alternariol in white maize. Regression analysis was used to determine the significant ($p < 0.05$) effect of the process variables on removal of mycotoxin from the maize. Results from this experiment indicated that hand sorting had the greatest effect on mycotoxin removal while flotation yielded the least effect. Further experimental results and implications are discussed in this paper.

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L27

Mycotoxin contaminations in grape must and wine samples resulting from automated optical grape-sorting process

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Grapes represent a favourable habitat for different microorganisms. Under convenient conditions the formation of various mycotoxins by moulds is possible and can result in the contamination of the berries and the prepared musts and wines. Concerning their detrimental effects on human health and the existing legal limits for some mycotoxins, absence or low concentrations are desired in food and feed. Grapes contaminated by moulds usually show a change in the appearance compared with healthy grapes. Therefore, the innovative possibility of grape-sorting by an automated optical system was tested for quality control, quality improvement and quality assurance of must and wine. Fifteen sorting experiments with different grape varieties from the Mosel wine region were realized in the years of 2010 and 2011. Besides the control fraction (unsorted berries), the resulting fractions of such a sorting process are a positive fraction (healthy berries), a negative fraction (rotten berries) and a free-run (naturally leaking juice from opened berries). These fractions were vinified in a standardized manner, so that the musts and the corresponding wines could be analyzed for 31 mycotoxins using our recently developed HPLC-MS/MS method with QuEChERS sample preparation for musts and wines. Results according to the mycotoxin occurrence and concentrations in musts and wines will be presented and discussed. In this study, automated optical grape-sorting was successful concerning the reduction of certain mycotoxin concentrations in musts and wines and provides an innovative possibility for a more effective quality assurance and quality improvement during winemaking, thereby making a major contribution for consumer protection.

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L28

Mycotoxin degradation in wheat straw by soil faunal communities under field conditions - An ecosystem service for soil health

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Mesocosm-field studies were conducted to demonstrate that deoxynivalenol (DON) contamination of wheat straw is substantially reduced in the presence of soil faunal communities in a practical cropping system. In 2011 and 2013, mesocosms were established in the topsoil of a winter wheat field located in Northern Germany. Soil fauna in different combinations (earthworms (E), E + collembolans (C), E + nematodes (N), E + C + N and a non-faunal treatment) was exposed to artificially *Fusarium culmorum*-infected wheat straw highly contaminated with DON (2011: 318.56 mg kg⁻¹; 2013: 40.97 mg kg⁻¹). Wheat straw with a low concentration level of DON (<2.54 mg kg⁻¹) served as control. After an experimental time span of 4 and 8 weeks, the DON concentration in residual straw and in soil was determined by using a competitive ELISA test kit. In both experimental years the initial DON concentration decreased in all treatments after 4 weeks, whereas the highest reduction was determined throughout the faunal treatments (2011: 97-99%, 2013: 78-90%). The reduction of initial DON concentration in the non-faunal control treatments was significantly lower (2011: 87%, 2013: 68 %). After 8 weeks a further decline of DON concentrations was measured in all treatments containing soil fauna (2011: 46-88%, 2013: 67-85 %), whereas the highest reduction was found in mixed treatments due to faunal interaction. DON contents in residual straw increased in the non-faunal treatments during the final four weeks in both experimental years. In contrast, the concentration of DON in soil of the mesocosms was below quantification limits (<0.037 mg kg⁻¹) throughout all treatments in 2011 and 2013, respectively. Our results clearly demonstrate that earthworms, collembolans and nematodes contribute in an interactive manner to a sustainable control of *Fusarium*-related contaminants in wheat straw thus reducing the risk of plant diseases and environmental pollution from as an important ecosystem service for soil health.

L29

Evaluation of atoxigenic strains of *Aspergillus flavus* for reduction of aflatoxin contamination of maize on commercial farms in Texas, USA

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Aflatoxin is a recurring problem in maize-production areas of central and southern Texas. In some years, 85-90% of maize samples exceed the US food-grade threshold of 20 ppb and levels exceeding 500 ppb are not uncommon. AF-36 and Afla-Guard are commercial preparations of different, non-aflatoxigenic strains of *Aspergillus flavus* that are labeled in Texas for aflatoxin control on maize. These products consist of the fungal strain carried on cereal grains and are applied just prior to flowering at a rate of 11.2 to 22.4 kg/ha. The objective of this research was to evaluate the effectiveness of both atoxigenic strains on commercial farms. In all experiments, the products were applied to the top of the plants by hand at a rate of 11.2 kg/ha. Replicates were 8 rows by 30 m long and there were four replicates per treatment. Treatments were separated by 30 m. The farmer's combine was used to obtain a steady stream grain sample of 2.2 to 4.5 kg, which was ground using a Romer mill and analyzed using the Vicam system. In all eight experiments, the average aflatoxin level of an atoxigenic treatment was less than that of the control, but in four of those experiments, the average levels of contamination of the controls were low, ranging from 4-5 ppb. In Ellis county, an Afla-Guard treatment applied at V6 to V9 had significantly ($P=0.05$) lower aflatoxin (126 ppb) than the control (340 ppb). In Hill county, average aflatoxin levels with Afla-Guard and AF-36 treatments ranged from 56 to 60 ppb, which were not significantly ($P=0.05$) less than the control (161 ppb). In Jackson county, both treatments had aflatoxin ranging from 0-1 ppb, which was significantly ($P=0.05$) less than the control (60 ppb). In Nueces county, Afla-Guard-treated maize had 2 ppb aflatoxin, which was significantly ($P=0.05$) lower than the control (31 ppb). There was lower aflatoxin with an early (V5) application in Hill county, but not in Nueces county.

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L30

Zearalenone detoxification and evolution of lactonase activity in fungi

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The resorcylic acid lactones are bioactive compounds mainly produced by fungi of the *Hypocreales* order. Among these, zearalenone (produced mainly by *Fusarium graminearum* and *F. culmorum* cereal pathogens) is one of the most impactful compounds (due to its accumulation in food and feed). In nature, zearalenone biosynthesis is retained due to significant fungistatic effects of the mycotoxin. In keeping with this, the ability to detoxify zearalenone is a considerable adaptive advantage for competing fungi (among them the mycoparasitic species of *Trichoderma* and *Clonostachys* genera).

The results of screening a diverse collection of 79 *Trichoderma* and *Clonostachys* isolates, for zearalenone lactonase homologs, demonstrate the presence of functional enzyme in divergent *Hypocreales* species (*Clonostachys rosea*, *C. catenulatum*, first finding of lactonase in *Trichoderma aggressivum*). Likewise, in vitro transcript profiling and assessments of toxin quantity point to divergent strategies of zearalenone detoxification (rapid, induced expression of lactonase vs gradual transcript accumulation). The parallel in silico experiments (phylogeny reconstruction, homology modelling) provide additional evidence for ancient origins and conserved features (active site residues) shaping the resorcylic acid lactonase activity within filamentous *Ascomycota*.

References:

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L31

***Stachybotrys chartarum* chemotype S in dried culinary herbs**

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Stachybotrys (S.) chartarum is a cellulolytic mould, which grows on wilting plants but also on building materials (Jarvis BB et al. (2000) *Stachybotrys*: An unusual mold associated with water-damaged buildings. *Myc Res* 16:105-108). Only chemotype S is able to produce macrocyclic trichothecenes. These toxins cause severe illnesses in horses (Forgacs J and Carll WT (1962) *Mycotoxicosis*. In: Brandly CA, Jungherr EL (eds) *Advances in Vet Sci*, vol 7. AP, New York:273-382) and are regarded as responsible for severe pulmonary haemorrhages in infants (Dearborn DG et al. (2002) *Clinical profile of 30 infants with acute pulmonary hemorrhage in Cleveland*. *Pediatrics* 110:627-637).

The role of *S. chartarum* in food is hardly studied. Spices are known to serve as vectors for microorganisms into food products. Culinary herbs represent a good habitat for cellulolytic moulds. Therefore we examined 80 samples of dried culinary herbs (marjoram, oregano, thyme and savory) for their overall mycobiota with emphasis on the isolation of *S. chartarum* chemotype S.

A total of 50 *Stachybotrys* spp. were isolated and further characterized by MTT- cell culture test, ELISA, and LC-MS/MS. Fifteen isolates were additionally identified by sequencing (Andersen B et al. (2003) *Molecular and phenotypic descriptions of *Stachybotrys chlorohalonata* sp nov and two chemotypes of *Stachybotrys chartarum* found in water-damaged buildings*. *Mycologia* 95:1227-1238). Five isolates proved to be highly cytotoxic in the cell culture assay. The production of macrocyclic trichothecenes was confirmed by ELISA and LC-MS/MS. All these five toxic isolates were identified as *S. chartarum* chemotype S by sequencing. The ten nontoxic isolates were found to belong to the closely related species *S. chlorohalonata*. These results show that culinary herbs must be regarded as a vector of *S. chartarum* chemotype S into food products.

L32

Distribution of *Fusarium* mycotoxins and their masked forms in wheat mill fractions

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Mycotoxins are the most important food and feed contaminants worldwide. Therefore, maximum limits in grain and grain products have been set for the frequently occurring mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) in the EU. In addition to DON and ZEN a variety of their derivatives, so-called masked mycotoxins, may co-occur in cereal and cereal based products. Nowadays they are of interest since these substances contribute to a relevant degree to the overall exposure of humans and animals with mycotoxins (De Boevre M, Di Mavungu J D, Landschoot S (2012) Natural occurrence of mycotoxins and their masked forms in food and feed products. World Mycotoxin Journal 5:207-2). As nearly no information on the distribution of the masked mycotoxins in milling fractions are available, these toxins cannot be included in any recommendation for secure flour production, yet. Therefore, the aim of the present study was to analyze the distribution of DON, ZEN and the main conjugates DON-3-glucoside (D3G), 3-Acetyl-DON (3Ac-DON), 15-Acetyl-DON (15Ac-DON) and zearalenone-14-sulfate (Z14S) in naturally contaminated wheat lots. For DON and its glucoside an almost uniform distribution over all milling fractions has been determined. The bran and the endosperm contribute nearly equally to the DON and D3G load. For ZEN and Z14S an increased proportion in the outer fiber-rich areas was detectable. The relative mass fraction of D3G based on the free DON has been determined to a range of 2.9% to 11.2%, while the relative mass portion of Z14S based on the free ZEN is higher (between 13.9% and 343.3%). The acetylated DON derivatives add only about 3% to the “total-DON“ content, even in the highest contaminated mill stream flours. The results show that an efficient reduction of mycotoxin contamination can be achieved by flour milling technology methods for ZEN and Z14S, but not for DON. As an overall result, the relevance of masked mycotoxins for ZEN can be ranked before DON.

L33

Fate of mycotoxins in the cornmeal processing from caryopsis to pre-cooked porridge

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Corn and maize are major and important crops consumed as food and feed. Mycotoxins, mainly fumonisins and aflatoxins, are a crucial problem concerning food products based on maize grain. In the last decade, studies focusing an aflatoxin contamination in foodstuffs indicate that corn is a main contributor to AFB1 exposure. Fumonisin may cause a variety of diseases in animals, as well as hepatocarcinogenic, hepatotoxic, nephrotoxic and cytotoxic effect in mammals. Moreover, there is evidence of a high incidence of human esophageal cancer associated with FB1 exposure. The problem of fumonisin contamination is further complicated by the fact that hydrolyzed forms and degradation products were found in thermally treated products but also in mild-treated products, at the same time possible modifications of mycotoxin structure by interaction with other food components (masked forms) may take place. The main objective of this study was to improve the comprehension of the influence of different treatment on fumonisin and aflatoxin occurrence in samples collected along the different production stages of a cornmeal industrial plant: pre-cleaned and cleaned corn, pre-cooked and cooked broken corn, pre-cooled and cooled corn-flake, pre-cooked flour “fumetto”, pre-cooked porridge, germ and middlings. Mycotoxins content was determined by HPLC-MS/MS analysis combined with adequate protocols for their extraction and clean-up. Results showed that no masked fumonisins were detected; higher amounts of contamination were detected in middlings (probably due to the presence of pericarp), while pre-cooked porridge was lower than the other fractions. All the analyzed samples intended for human consumption showed levels of contamination below the legal limits reported in EC Regulation 1126/2007; on the other hand, findings obtained for byproducts should be taken into consideration to assess feed safety and quality.

L34

Molecular quantification and genetic diversity of toxigenic *Fusarium* species in northern Europe and Asia

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The highest DON and T-2/HT-2 toxin levels in northern Europe have been found in oats. About 12-24% of Finnish oat samples in 2012 contained >1750 ppb of DON. *Fusarium graminearum* is the most important DON producer in northern Europe and Asia and it has been replacing the closely related *F. culmorum* in northern Europe. The 3ADON chemotype of *F. graminearum* dominates in most northern areas, while the 15ADON chemotype of *F. graminearum* is dominating in Central and southern Europe. We suggest that the northern European population may be specialized more to oats than the southern European population. No clear correlation was found between *F. culmorum* DNA and DON levels. DNA levels of *F. graminearum* were in all cases in agreement with DON levels, when DON was measured by GC-MS. When the RIDA®QUICK SCAN kit results (DON) were compared to DNA levels of *F. graminearum*, the variation was much higher. The homogenization of the oats flour by sieving seems to be connected to this variation.

There is also a significant correlation between the combined T-2 and HT-2 mycotoxin levels and the combined levels of *F. sporotrichioides* and *F. langsethiae* levels in Finland. But there are not enough morphological characters to clearly separate all isolates of the European species *F. langsethiae* from the northern Asian species *F. sibiricum*. The long TG repeat in the ribosomal IGS region is the only known DNA sequence that has been used to design a species-specific primer pair for identification of *F. sibiricum* isolates. Another way to identify *F. sibiricum* is to use a combination of a *F. sporotrichioides*-specific primer pairs, which give a positive signal both with *F. sporotrichioides* and *F. sibiricum* isolates, and *F. langsethiae*-specific primer pairs, which give a clear positive signal only with *F. langsethiae* isolates. *F. langsethiae* is a European species, while the main distribution of *F. sibiricum* is in northern Asia. The identification of the first a *F. sibiricum* isolate from Iran and the first *F. langsethiae* isolate from Siberia is confirmed by both molecular and morphological methods. A single isolate of *F. sibiricum* is also found in Norway. In conclusion, the actual distribution of *F. sibiricum* may be much larger than what is presently known.

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L35

A comprehensive definition of modified and other forms of mycotoxins including “masked” mycotoxins

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The rationale behind the new definition presented here was an ongoing discussion about the term "masked mycotoxins". In 2011, the International Life Science Institute (ILSI) has adopted the following definition: "Mycotoxin derivatives that are undetectable by conventional analytical techniques because their structure has been changed in the plant are designated masked mycotoxins".

As the term “masked mycotoxins” encompasses only conjugated mycotoxins generated by plants and no other possible forms of mycotoxins and their modifications, we here propose for all these forms a systematic definition consisting of four hierarchic levels. The highest level differentiates the free and unmodified forms of mycotoxins from those being matrix-associated and from those being modified in their chemical structure. The following lower levels further differentiate in particular “modified mycotoxins” into “biologically modified” and “chemically modified” with all variations of metabolites of the former and dividing the latter into “thermally formed” and “non-thermally formed” ones.

To harmonize future scientific wording and subsequent legislation, we suggest that the term “modified mycotoxins” should be used in future and the term “masked mycotoxins” to be kept for the fraction of biologically modified mycotoxins that were conjugated by plants.

L36

The approach of the European Union Reference Laboratory to promote reliable measurements

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The European Union Reference Laboratory (EURL) for mycotoxins was established in 2006 and acts as key player together the National Reference Laboratories (NRLs) to assure the harmonisation of mycotoxin measurements in food and feed. One of the tasks of the EURL are the conduction of comparative tests/proficiency-tests (PTs) with NRLs and associated reference laboratories. These PTs primary aim at benchmarking laboratory performance of NRLs.

However with a suitable catalog of questions it could be shown that identifying bottle necks and pit falls in analytical procedures is possible. Further the promotion of robust procedures for future method standardisation efforts is possible. This additional information gives important feedback to the participants, helpful implementing effective root-cause analysis for improment and planning sound corrective action plans. In the majority of cases improvment is verified by follow-up Pts.

The presentation will give an overview on the insights the EURL has gained with its NRL network over the years to promote reliable analytical measurements and what laboratories participating in PTs should consider in the selection of PT schemes.

Abstracts of Posters

P1

A multi-mycotoxin UHPLC-MS/MS method for the detection, quantification and identification of mycotoxins in milk replacer

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Veal calves are predominantly fed liquid milk replacers, containing vegetable protein sources such as soy, corn and wheat, implying risk of mycotoxin contamination. In order to perform a survey of the exposure of veal calves due to feeding milk replacer, an ultra-high pressure liquid chromatographic-tandem mass spectrometric (UHPLC-MS/MS) method for the analysis of 12 mycotoxins in milk replacer was developed. The mycotoxins considered were: aflatoxins, alternariol, alternariol monomethyl ether, deoxynivalenol (DON), 3-AcDON, 15-AcDON, fumonisins, ochratoxin A, T-2 toxin and zearalenone.

The UHPLC-MS/MS system used consisted of an Acquity UHPLC (Waters) chromatographic system coupled to a Xevo TQ-S MS triple quadrupole system (Waters). Chromatographic separation was performed on an Acquity CSH Fluoro Phenyl column (1.7 μm , 2.1 x 150 mm, Waters). A gradient (17.0 min) was applied using as mixture of H₂O + 0.3% acetic acid (A) and methanol (MeOH) + 0.3% acetic acid (B) as mobile phase. Mycotoxins were extracted from the matrix by means of a twofold extraction procedure: a first extraction step with MeOH followed by a second extraction step with acetonitrile/H₂O/acetic acid (79/20/1). ¹³C-standards (of DON, 3-AcDON, T-2 and ZEN) were used as internal standards. The validation parameters specificity, linearity, apparent recovery, repeatability, reproducibility, specificity, limit of detection (LOD) and quantification (LOQ) were evaluated for the method developed. For determining the apparent recovery, repeatability and reproducibility, two concentration levels were considered. The criteria of linearity and specificity were fulfilled for all mycotoxins. The average apparent recovery varied between 85 and 107%, which is in agreement with the ranges set in Commission Decision 2002/657/EC. Average repeatability and reproducibility ranged between 0.9 and 10.5% and between 1.5 and 12.8%. The LOD and LOQ values were within the range of 1-89 $\mu\text{g}/\text{kg}$ and 2-171 $\mu\text{g}/\text{kg}$, respectively.

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P2

Comparison of a tenuazonic acid enzyme immunoassay with SIDA-LC-MS for the determination of tenuazonic acid in sorghum and infant food

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L-tenuazonic acid ((5S)-3-acetyl-1, 5-dihydro-4-hydroxy-5-[(1S)-1-methylpropyl]-2H-pyrrol-2-one), TeA, is a tetramic acid derivate commonly listed within the group of *Alternaria* mycotoxins. The European Food Safety Authority (EFSA) evaluated the toxicological potential of TeA by following the threshold of toxicological concern (TTC) approach yielding TTC value of 1,500 ng TeA/kg body weight per day. Recently, high amounts of TeA were detected in sorghum and sorghum-based infant food. In this study, the results of a comparison study between an immunochemical method for TeA (EIA) with a LC-MS/MS method (stable isotope dilution assay, SIDA) is described.

A total of 28 selected sorghum-based food samples (infant cereals, n=16; sorghum grains, n=12) from the German market were analysed in this study. Samples had first been analysed by SIDA, and then were selected for the comparison study to cover a wide concentration range. These samples were then analysed independently by EIA. Mean recoveries for TeA in the SIDA were 99.7% in solid matrices with low water content (detection limit: 1 µg/kg). In the EIA system, mean recoveries for sorghum (200-600 µg/kg) were 47.6% (detection limit: 27.2 µg/kg) and mean recoveries for infant cereals (400-600 µg/kg) were 96.9% (detection limit: 224 ng/g).

In the SIDA, 100% of the samples contained TeA above the detection limit, in a concentration range of 6 – 584 µg/kg (mean: 113 µg/kg). In the TeA EIA, approximately half of the samples (56.3%) of the infant cereals contained TeA in a concentration range of 286 - 644 µg/kg (mean: 452 µg/kg) and 50% of the sorghum samples contained TeA in a concentration range of 31.9 - 192 µg/kg (mean: 95.6 µg/kg).

The results show that, although the EIA is less sensitive than the LC-MS method, it may still be suitable to detect TeA in sorghum material at levels exceeding 200-300 ng/g, and enables quantification at levels >500 ng/g. At the moment it is questionable at which levels TeA should be regulated in infant foods, because toxicity data are insufficient for a thorough risk assessment. However, the TeA EIA could be useful to identify batches of sorghum with higher levels of TeA, and thus enhance the analytical repertoire for this toxin in food hygiene.

P3

HPLC-HRMS in combination with software tools: A new strategy for secondary metabolite screening of *Fusarium fujikuroi*

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Secondary metabolites of moulds can have both advantages and disadvantages for mankind. They can serve as pharmaceuticals but can also be highly toxic. Therefore it is important to screen the metabolite-spectrum of fungi in order to prevent health risks and to find new potential bioactive components. *Fusarium fujikuroi* is an ascomycetous fungus which normally infects rice. On rice, it is responsible for the bakanae disease. The recently sequenced genome of the *Fusarium* strain revealed 45 potential secondary metabolite gene clusters. Since there are less metabolites than identified gene clusters, there is a high potential for the discovery of new secondary metabolites. However, most of these gene clusters are not expressed under standard laboratory conditions. Therefore, genetic manipulations of cluster or regulatory genes are one possibility to identify new products.

Within this study, the application of different software tools for the data processing of HPLC-HRMS raw data is described. By comparing the processed data of several mutant strains with the fungal wild type, differences and similarities can easily be found.

Mutants with knockout or overexpression of genes encoding either global regulators or pathway-specific enzymes were used for comparison with the corresponding wild type. Differences in the secondary metabolite profile occur due to these genetic interferences. By measuring the culture filtrates of the strains via HPLC-HRMS and analysing the occurring differences with various software tools, new secondary metabolites have been identified. One of them is a new bikaverin intermediate discovered in the knockout strain of a gene involved in the bikaverin biosynthesis. Bikaverin is a red pigment produced by *F. fujikuroi* under low-nitrogen conditions which presumably protects the fungus against environmental stress, for example UV light.

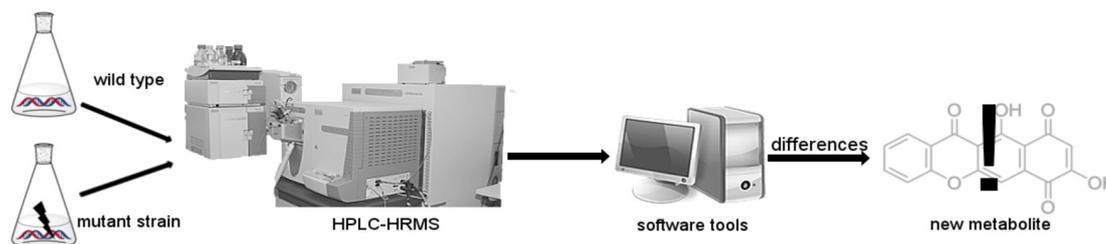


Figure. Workflow of the secondary metabolite screening

This work was supported by the NRW Graduate School of Chemistry.

P4

Report on the proficiency test about the *Alternaria* toxins tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and altenuene (ALT) in food

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Alternaria toxins (ATs) are mycotoxins that are produced by moulds of the genus *Alternaria* (black moulds), which preferably infect cereals, fruits and vegetables such as tomatoes. In 2003, the BfR evaluated ATs in food with regard to a potential risk for human health. At present, there is a paucity of data concerning the occurrence of *Alternaria* toxins in food and feedstuff. The analysis of ATs is currently lacking standardised validated analytical methods. There are several “in-house” validated methods, most of which are based on HPLC-MS/MS. To evaluate the comparability of methods used for the determination of ATs the National Reference Laboratory for Mycotoxins at the Federal Institute for Risk Assessment in Berlin has organised a first inter-laboratory comparison among laboratories in Germany, Switzerland, Austria and Belgium concerning *Alternaria* toxins. The proficiency test focused on the determination of TeA, AOH, AME, TEN and ALT in tomato juice. Seventeen participants were registered for the test. Testing material consisted of three test items with different concentration levels of TeA, AOH, AME, TEN and ALT based on commercially purchased tomato juice. All samples were evaluated for homogeneity. The stability of the toxins in the frozen reference material was confirmed over the whole proficiency test period. The test items were dispatched to the participants in November 2013. The participants were free to choose a method for the sample preparation and quantification of the toxins. They were invited to report their results as well as details corresponding to the method conditions by March 2014. Laboratory results for the ATs were rated with z-scores in accordance with DIN ISO 38402 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Data obtained from the current proficiency testing are presented and discussed in relation to the different methods applied.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011. Scientific Opinion on the risk for animal and public health related to the presence of *Alternaria* toxins in feed and food. EFSA Journal 2011; 9(10):2407

BfR (2003) *Alternaria*-Toxine in Lebensmitteln. Stellungnahme des BfR vom 30. Juli 2003

P5

Determination of deoxynivalenol and major conjugates in cereal samples using a solvent free extraction/clean-up step coupled online to HPLC-PCD-FLD

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The potential reformation of regulated mycotoxins from their masked forms during mammalian digestion raises similar health concerns as their parent mycotoxins. Therefore their occurrence, human exposure and toxicity are focus to investigation (De Boevre et al. 2014, Berthiller et al. 2013). The Codex Committee on Contaminants in food proposed to take other relevant deoxynivalenol (DON) derivatives into account when drafting maximum levels. The European Commission welcomed the initiative to monitor for relevant DON derivatives such as 3-AcDON and 15-AcDON and where possible DON-3-G.

Employing a progressive clean-up procedure (Seidler, 2007), we have developed an expedite method for the determination of deoxynivalenol and major conjugates consisting of an inline immunoaffinity column clean-up coupled to high performance liquid chromatography/post-column derivatization. This method has the merit of a high detection capability due to complete transfer of the purified analytes for fluorescence detection. The absence of any organic solvent in the sample preparation step avoids evaporation and reconstitution of the extracts. The method was applied for the determination of DON and its conjugates in barley, wheat and maize in the range of 10-1000 μgkg^{-1} of DON, 3-300 μgkg^{-1} of DON-3G and 15-AcDON and 1-100 μgkg^{-1} of 3-AcDON. Recoveries were consistently above 90% and precision was below 10% RSD. Nevertheless, a judicious selection of the immunoaffinity columns has to be made as only one brand tested was able to cross-react with the 3 DON conjugates. This poster will show how with attention to analytical details a rather simple method for the monitoring of DON conjugates in cereals was developed. Sixteen maize, wheat and barley samples were analysed revealing variable levels of DON conjugates.

P6

LC-MS/MS determination of *Alternaria* toxins in various food products

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The data to carry out a risk assessment for *Alternaria* toxins are not adequate (EFSA (2011) Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food (EFSA Journal 9 (10): 2407; Lorenz N, Klaffke H-S, Kemmlin S, Itter H, Lahrssen-Wiederholt M (2012) Aktueller Sachstand zum Thema *Alternaria*-Toxine aus Sicht des gesundheitlichen Verbraucherschutzes. J. Verbr. Lebensm. 7: 359-365). Therefore it is necessary to collect further data about occurrence and amount of these toxins in food.

The analytical method for determination of alternariol (AOH), alternariol monomethyl ether (AME) altenuene (ALT) and tentoxin (TEN) includes the extraction with acetonitrile/methanol/water (pH 3), followed by clean up with SPE and solvent change (methanol/water) before injection into the LC-MS/MS (Reinhold L, Bartels I (2007) Bestimmung von *Alternaria*-Toxinen in Lebensmitteln. Labor Praxis 31. Jahrgang: 62-64). Tenuazonic acid (TEA) is determined after derivatisation with 2,4-Dinitrophenylhydrazin (Siegel D, Rasenko T, Koch M, Nehls I (2009) Determination of *Alternaria* mycotoxin tenuazonic acid in cereals by HPLC-ESI-MS after derivatisation with 2,4-dinitrophenylhydrazine. J. Chromatogr. A 1216: 4582-4588).

The present work shows the sample preparation and the results of the analysis of tomato puree, tomato juice, dried tomatoes, sunflower seeds, millet seeds, fruit beverages and spices. Tenuazonic acid was found in the named food in high levels except for fried mashed potatoes. Alternariol was only present in millet seeds, tomato puree and dried tomatoes. Alternariol monomethyl ether was found in sunflower seeds, tomato puree, dried tomatoes and paprika spice. Altenuene was detected in sunflower seed and tomato puree, tentoxin in sunflower seeds and dried tomatoes.

P7

Analysis of various mycotoxins using a solid phase extraction sorbent based on Molecularly Imprinted Polymers

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Mycotoxins are toxic secondary metabolites produced by organisms of the fungi kingdom which can commonly be found in food and feed. When regulated, their concentration cannot exceed a certain amount which is very low in most cases due to health risk for consumers. This poster shows results obtained for two solid phase extraction (SPE) clean-up methods based on the use of Molecularly Imprinted Polymers (MIP). MIPs are affinity columns made with very stable polymers to aqueous or organic solvents as well as temperature. These cost-effective products are widely used for clean-up and preconcentration applications.

The first method has been developed for the analysis of deoxynivalenol (DON) mycotoxin (a.k.a Vomitoxins) and its derivatives 3-Acetyl DON and 15-Acetyl DON on cereals. These B trichothecene mycotoxins predominantly occur in grains such as wheat, barley, oats, rye, and maize where they are very commonly found. So it is regulated in Europe and USA. The method developed has been evaluated with several extractions protocols and shows high recovery yield and excellent clean-up

The second method enables the analysis of multimycotoxins on cereals. Indeed a broad family of mycotoxins, aflatoxins, ochratoxin A, fumonisins, zearalenone, HT-2 and T2 toxins could be analyzed by this fast, robust and effective clean-up process. High recovery yields were obtained. These methods can be used prior to ELISA analysis or LC-MS/MS detection.

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P8

Aflatoxin B/G/M1 and ochratoxin A analysis: fully automated from the raw extract to the chromatogram at ultimately low limits of quantification

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Mycotoxins are highly toxic metabolites from various fungi. The amount of certain mycotoxins are strictly regulated in food and feed and are under observation to ensure public health.

The extraction of such toxins is often done using methanol/water or other organic solvents. The clean-up of the toxins for sensitive analysis is a labor intensive aspect in the sample preparation and analysis. The strong regulation and the low level of tolerated toxins in various food matrices especially those which are dedicated for diet food or baby food is a challenge, because the concentration of the eluted sample is a time consuming and error-prone process.

A new technology offers direct sample clean-up and online injection of the prepared sample. The technology is based on immune affinity technology combined with a thermal elution of the toxin from the immune affinity column and direct injection into the analytical HPLC. Furthermore, the time consuming aspects of sample loading and subsequent solvent exchange are dramatically reduced.

The direct injection of the eluate allows increasing the sensitivity down to 10 ppt (ng/kg) for aflatoxins B/G. The implementation of AflaCLEAN M1 SMART performs the analysis of milk samples in a high throughput mode as low as 5 ppt (ng/kg). As a consequence of the gain in sensitivity significantly reduced sample volumes or higher dilution of the crude extracts, which reduces matrix interferences dramatically, are possible.

The sensitivity of the ochratoxin A analysis is better than 30 ppt (ng/kg). Furthermore, the total process for sample analysis takes less than 25 min per samples.

All regulated matrices (e. g. cereals, nuts, spices, baby food...) can be processed; the obtained chromatograms are easy to evaluate and exhibit no matrix dependence.

This technology is dedicated for a 24/7 sample preparation and in combination with the online injection into any HPLC-FLD or LC-MS/MS increases the sample throughput as high as gt 500 samples/week.

P9

Development and validation of rapid analysis for twelve mycotoxins in cereal based foods using immunoaffinity clean-up and a single quadrupole mass detector

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Mycotoxin contamination represents the highest number of notifications under the EU Rapid Alert System for Food and Feeds with up to 25% of the world's cereal grains being potentially contaminated. The majority of mycotoxins are extremely stable and can easily enter the marketplace.

Here we report the development and validation of a screening procedure based on the use of a multitoxin immunoaffinity clean-up (Mycotoxin1+) coupled to ultra-performance liquid chromatography (UPLC) and single quadrupole mass detector (ACQUITY QDa). The method is applicable for the routine detection of mycotoxins including aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A, fumonisins (FB1 and FB2), deoxynivalenol, zearalenone and T-2 /HT2. These mycotoxins are currently regulated within European Legislation. Although not regulated yet, attention was paid to the occurrence of nivalenol another Fusarium toxin that frequently contaminates cereals.

Wheat, maize and processed cereal samples were extracted using VICAM (Mycotoxin1+) immunoaffinity columns, where no significant matrix effects were observed. Excellent repeatability was achieved in respect to retention time, peak shape and response. Good linearity was determined for all mycotoxins with limits of detection achieved well below the permitted levels. Replicate injections of low concentrations in matrix provided excellent repeatability and accurate quantification of all mycotoxin analytes.

The robust, repeatable and selective performance will be presented for the 12 mycotoxins in a variety of commodities. The ACQUITY QDa detector provides high quality mass spectral data following Mycotoxin1+ clean up, improving selectivity over optical detectors (UV and fluorescent) routinely used in the food industry. The advantage offered by mass detection over these conventional methods will be highlighted, including enhanced analytical performance, ease of use and flexibility, allowing for multiple methods to be consolidated into a single method.

P10

Multivariate optimization of a simultaneous extraction method for 10 mycotoxins from wheat and oats

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Mycotoxins may cause health problems to humans and animals. Cereals such as wheat and oats are a staple food to millions of people and their contamination poses a big risk to health and economic losses. Thus, there is a need to develop analytical methods for rapid, sensitive and accurate determination of these mycotoxins. Extraction of mycotoxins is the most laborious and time consuming step in the analysis. Multi-extraction methods may greatly shorten the analytical procedures but the challenge lies in the varying physicochemical properties of the mycotoxins.

The aim of this study was to develop by statistical means a fast and simple simultaneous extraction method to extract aflatoxin B1, deoxynivalenol, fumonisin B1, fumonisin B2, HT-2 Toxin, ochratoxin A, roridin A, sterigmatocystin, T-2 Toxin and zearalenone from oats and wheat.

A Box-Behnken design of experiment and response surface methods were successfully used in developing two multi-extraction procedures. For the evaluation of ion suppression or enhancement during the ESI-LC-MS/MS analysis, matrix effect profiles were investigated. In addition, matrix effects were quantified by comparing the results of the analysis of three sets of spiked and unspiked samples. All mycotoxins were affected by strong matrix effects in wheat and oat.

A single method to extract all mycotoxins in wheat and oats was not possible due to their strong differences. The optimal extraction parameters were 80% methanol in water, 52 °C at pH 7 and 40% methanol in water.

The design of experiment approach is highly recommended because a lot of time and costs were saved by carrying out a few planned experiments. The evaluation and compensation of matrix effects is always necessary.

P11

LC-MS strategy for effect-based analysis of toxins in complex samples by on-line coupled enzyme-assay

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Health risk assessments at potentially contaminated working or indoor environments are often limited. Routine analytical methods are focused only on known substances, while other harmful compounds remain undetected. With effect-based test procedures the identification and evaluation of the toxicological relevant compounds is possible. Here, we present an approach for simultaneous identification and characterization of unknown regulative active compounds by an on-line LC-MS enzyme assay. In figure 1 the regulative effect of a known inhibitor to the model enzyme acetylcholinesterase is demonstrated. The complex extract of a molded building material is spiked with the known alkaloid galantamine (black line) that inhibits the acetylcholinesterase-catalyzed degradation of the natural substrate acetylcholine (red line) to acetate and choline (blue line). Other unknown compounds do not influence the enzyme activity. The chosen enzyme assay is capable to determine regulative active compounds like mycotoxins. Aflatoxin B1 strongly influences the kinetic by inhibiting the enzyme. First scientific findings pointed out that for on-line assays protein adhesion and depletion are the main drawbacks of this approach. To overcome those problems miniaturization is a promising tool and it is recently under investigation. The different milestones for on-line enzyme assay and miniaturization for the detection of regulative active mycotoxins will be presented.

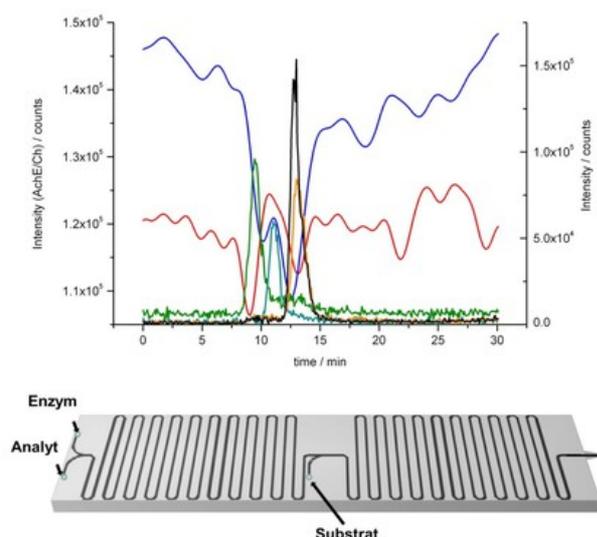


Fig. 1 a) LC-MS enzyme-assay chromatogram, b) Lab-on-chip solution of an on-line coupled enzyme-assay

P12

Development of an LC-MS/MS method for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulide

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Beauvericin (BEA) and the related enniatins (ENN) are secondary metabolites produced by different *Fusarium* species. These fungal metabolites are known as mycotoxins. The emetic toxin cereulide (CER) is produced by the bacterium *Bacillus cereus*. Although these toxins have a different origin, the striking structural and functional similarities should allow a combined detection in food matrices. Both fungal and bacterial toxins are cyclodepsipeptides with a high affinity for ammonium cations which can form ionophoric, lipophilic stable complexes. They act as toxic agents by forming membrane carriers for cations resulting in disturbances in the physiological cation level in the cell. In the area of food safety, the determination of the occurrence of these medium-sized cyclodepsipeptides in food and feed is imperative.

The aim of this project is to develop a method for the simultaneous determination of both BEA, the related ENN and CER in cereal-based food matrices such as pasta, rice, wheat and maize. The analytical method of choice for combined detection is ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Gradient elution was chosen to allow separation of the different toxins within a time frame of 9 min. The optimal LC and MS conditions for BEA, ENN and CER were determined using valinomycin as internal standard and the sample preparation method was developed. Based on these findings, a full validation of the method will be performed.

Keywords: Enniatins; Beauvericin; Mycotoxin; Cereulide; Food analysis; LC-MS/MS

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P13

A master-curve calibrated immunoassay for the quantitative detection of zearalenone in maize, wheat, feed and DDGS

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Zearalenone (ZON) is a non-steroidal oestrogenic mycotoxin produced by different *Fusarium* species that grows in moist and cool field conditions. ZON has been reported to occur in different cereals and their by-products. ZON presence in animal feed is frequently involved in reproductive disorders in pigs, cattle and sheep.

Among methods for routine screening of ZON in different matrices, ELISAs are good options thanks to their reliability, sensitivity and precision, but their cost-effectiveness is lacking if a few samples are analysed per session, due to the necessity to consume wells for the calibrators. The aim of the present work is the development of B ZERO ZON, a reliable immunoassay for the quantitative detection of zearalenone with a proper master-curve, which allows to run the “zero” standard and the samples, only. B ZERO ZON is a 15 minutes ELISA test kit with a master-curve range of 25-2000 ppb, extendable up to 10000 ppb.

Four matrices - maize, wheat, complete as well as complementary swine feed and DDGS (wheat and maize by-product of distillation, often used to produce animal feed) - were validated with the new ELISA.

Blank samples of maize, wheat, feed and DDGS were analysed to verify the assay specificity. The cut-off was set at 50 ppb for wheat, 30 ppb for maize, 65 ppb for feed and 90 ppb for DDGS. The Limit of Quantification (LOQ) was set at 50 for wheat and maize and 70 ppb for feed. The mean recovery for HPLC-analysed incurred samples was 96+10% (n = 10) for whole wheat, 85+25% (n = 24) for maize, 110+31% (n = 19) for feed and 94+30% for DDGS (n = 15). The mean intra-assay CV always turned to be < 5%, the mean inter-assay CV was < 15%. As a conclusion, B ZERO ZON showed good performances for all the investigated matrices.

P14

Improvement of a sample preparation method for the determination of deoxynivalenol, zearalenone and their metabolites in pig urine with LC-MS/MS - Optimization of the incubation conditions

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The *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZEN) occur at toxicologically relevant concentrations in cereals and maize. In animals, DON and ZEN were metabolized to de-epoxy-deoxynivalenol (de-DON), α - and β -zearalenol (α -, β -ZEL), zearalanone, α - and β -zearalanol. Further, conjugation with glucuronic acid takes place, whereby almost all toxins were found as glucuronides in pig urine. As glucuronides cannot be detected directly with the used LC-MS/MS method, they had to be hydrolysed with β -glucuronidase (β -Gluc) before analysis. Therefore, the aim of this work was to improve the incubation conditions for pig urine.

The samples were obtained from an animal feeding trial with pigs fed diets with graded levels of DON and ZEN. Pig urine samples with high toxin concentrations were pooled. In the original method 500 μ l pig urine was incubated with 80 μ l β -Gluc and 750 μ l sodium acetate buffer (pH 5.5) for 16h. Afterwards, solid phase extraction was performed for clean-up and the mycotoxins were analysed with LC-MS/MS. According to this method different times of incubation (0-16h), amounts of β -Gluc (0-120 μ l) and different pH values of the buffer (pH 5.5; 5.0) were tested.

The results showed that incubation without β -Gluc led to no quantifiable concentrations of ZEN and its metabolites. Also the de-DON concentrations were very low, whereas DON was quantified in mentionable concentrations. α -ZEL, β -ZEL and de-DON concentrations slightly increased with increasing amounts of β -Gluc. The incubation times 6 and 16h led to the highest mycotoxin concentrations. In contrary to that the ZEN concentration decreased with increasing amount of enzyme. The impact of the two buffers is not clear so far, but further examinations will be made. It has been shown, that higher toxin concentrations could be obtained with different changes of the three parameters, but compromises have to be made for the simultaneous determination of ZEN, DON and their metabolites.

P15

A quick, easy, cheap, effective, rugged and safe (QuEChERS) LC-MS/MS method for the determination of citrinin in red yeast rice products

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Red yeast rice is a fermentation product of rice with molds from the genus *Monascus*. Among others, it is used as a dietary supplement because it contains monacolin K which is said to have a positive influence on the LDL-cholesterol level. In accordance with commission regulation (EU) No. 432/2012, a health claim for red yeast rice products containing monacolin K has been authorized (Commission Regulation (EU) No 432/2012. OJ L 136. 25.02.2012. p-1). However, under certain conditions some species of *Monascus* are known to produce the mycotoxin citrinin. Based on this fact, the establishment of a maximum level for citrinin in red yeast rice products in the EU is currently being discussed.

To ensure consumer protection, convenient and reliable analytical quantitation methods are required. Recently rapid methods (with no or simplified clean-up steps) using high performance liquid chromatography (HPLC) and mass spectrometry (MS/MS) have become very popular in mycotoxin food analysis. A disadvantage of these methods is the co-extraction of matrix substances, which can disturb the quantitative analysis. That may also be the case with red yeast rice products, because they contain high amounts of pigments and are known to be difficult matrices.

A QuEChERS (Anastassiades M Lehotay SJ Stajnbaher D Schenck FJ (2003) J AOAC Int 86:412–431) based HPLC-MS/MS method has been developed to determine citrinin in red yeast rice products. Different clean-up sorbents, such as PSA (primary and secondary amines), GCB (graphitized carbon black sorbent), MgSO₄, CaCl₂, and their combinations have been investigated. The aim was to remove co-extracted matrix substances, thereby minimizing potential matrix effects in order to obtain good quantitation results. The clean-up sorbent combination finally chosen contained MgSO₄, GCB and CaCl₂. This resulted in adequately decoloured acetonitrile extracts and an acceptable recovery for citrinin in a spiked red yeast rice sample.

P16

Simultaneous determination of total aflatoxin, ochratoxin A and fumonisin using AOF MS-PREP® in conjunction with LC-MS/MS

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European legislative limits for total aflatoxin, ochratoxin A and fumonisins are in place for cereals and cereal products. There is a demand in the market for faster and less labour intensive tests. Immunoaffinity columns are rapidly becoming the routine standard method of choice for complying with regulatory mycotoxin analysis however there is a growing need for multi-mycotoxin analysis using a single extraction method. In response, R-Biopharm Rhone has produced a multi-toxin immunoaffinity column, AOF MS- PREP® enabling the isolation and concentration of aflatoxins B1, B2, G1, G2, ochratoxin and fumonisin in cereals and cereal products. Recoveries range from 78 to 106 % for maize and from 80 to 109 % for animal feed samples, with low % RSD when tested at European Legislative Limits. The advantages of this new immunoaffinity column are that only one sample preparation method and one single LC-MS/MS run is required for quantifying all four mycotoxins therefore having greater sample throughput and a reduction in the use of solvents and consumables.

P17

Validation of an automated system for aflatoxins in a range of samples using online immunoaffinity cartridges in conjunction with HPLC

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R-Biopharm Rhone have developed a patented, online affinity cartridge, which can be used prior to HPLC or LC-MS/MS. The cartridges are used together with the Symbiosis™ handling system and combine automated online sample application with quantitative analysis of aflatoxin B1, B2, G1 and G2. The affinity cartridge contains a monoclonal antibody that is specific for aflatoxins coupled to a hydrophilic polymer that can withstand high pressure.

Using the IMMUNOPREPT™ ONLINE AFLATOXIN cartridges, the sample application, washing and elution is performed online for up to a maximum of 12 samples before the cartridge is automatically removed and replaced with a new cartridge. The technology is a highly innovative automated analysis for aflatoxins which is highly specific, sensitive and rapid.

A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero. The use of an online affinity cartridge reduces labour, consumables and solvents, whilst improving traceability, accuracy and reducing human error.

P18

Determining experimental method to establish toxin binding capacity

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The contamination of feed with mycotoxins represents a worldwide problem for the animal industry. Aflatoxicosis, poisoning after ingesting aflatoxins (Af), causes severe animal pathology and economic losses. To alleviate these effects in animals, several clay mineral based adsorbents can be used. Clay minerals, including bentonites, are recognized to be effective in adsorbing mycotoxins, such as aflatoxins.

The EU Reference Laboratory for Feed Additives (EURL) approved a general European *in vitro* method for the determination of the aflatoxin B1 binding capacity (BCAfB1 value) of bentonite type clays from various origins (Regulation No 1060/2013 of 29 October 2013 concerning the authorization of bentonite as a feed additive for all animal species). As described in this European method, an adsorption test is carried out in a buffer solution at pH 5.0 with an AfB1 concentration of 4 mg/L and 0.02 % (w/v) toxin binding material. The applied test uses these intensified conditions (i.e. a low adsorbent and a high toxin concentration) to differentiate the adsorption capacities of different tested adsorbents.

Since the EURL method leaves room for analytical interpretation of various parameters, including physical parameters related to brand dependent geometry of equipment, initial conditions for stock solutions or parameters related to chemical compositions of recipients, the *in vitro* AfB1 adsorption percentages could be influenced. Big variations are observed for those parameters that accumulate up to 10% difference in binding capacities for AfB1 adsorption in a single bentonite sample. Identical binding material samples were sent to an independent analytical lab to determine for the AfB1 adsorption according to the EURL certified method. The received results were comparable with our findings, which confirmed that the interpretation of the EURL approved method could lead to an overall 10% variation in binding capacities.

P19

Strategies to produce antisera against sterigmatocystin by different sterigmatocystin-protein conjugates

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Sterigmatocystin (STC) is a mycotoxin structurally related to the aflatoxins. It is produced by different species within the genus *Aspergillus*, but it has also been detected in other fungal genera. It is somewhat less toxic than the aflatoxins, but still has been classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC). STC occurs as a preharvest or postharvest natural contaminant in many types of foodstuff, especially in cereals and in cheese. Although chromatographic methods are available for STC, a rapid screening test like for aflatoxins does not exist. Immunochemical approaches described so far require chemical conversion of STC into its hemiacetal before detection, which is due to problems of immunogen synthesis, but is also prohibitive for a convenient screening procedure. The objective of the present study is the production of novel STC immunogens and their use for the generation of polyclonal antibodies against STC.

STC was conjugated to keyhole limpet hemocyanin (KLH) by two different methods, the Mannich condensation (MC) and the carbonyldiimidazole method (CDI), respectively. Furthermore the carbonyldiimidazol-method was used to produce a STC-horseradish peroxidase (HRP) -conjugate as the labelled antigen. Rabbits were immunised either with with the STC-MC-KLH and the STC-CDI-KLH. Each rabbit was restimulated three times after basic immunisation. Blood was collected every four weeks. The immune response as the specific antibody titer was screened in a direct and indirect EIA by using different solid phase antigens. Additionally, ultrasensitive antibodies against aflatoxin B₁ were used to test the cross-reactivity with different STC-solid phase antigens (BSA and glucose oxidase conjugates of STC). First results indicate that the STC-MC-KLH yielded only a weak and non-specific immune response. Results from the immunization experiments with the STC-CDI-KLH will be presented in this contribution.

P20

Combined multianalyte-IAC column clean-up of regulated mycotoxins in one common sample preparation step prior to LC-MS/MS analysis

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Introduction: In food and feed control, the determination of mycotoxins plays a prominent role world wide. Normally, a single mycotoxin is cleaned up in one step using an immunoaffinity column. That does not fit to the multianalyte capability of mass spectrometry. B-TeZ IAC Combi AOZDFTM now combines the advantages of high efficient enrichment potential of an IACcolumn with multianalyte detection of mass spectrometer. B-TeZ IAC Combi AOZDFTM is validated for the simultaneous determination of the strictly regulated mycotoxins (aflatoxins, ochratoxin, zearalenone, deoxynivalenol, fumonisins and T2-Toxin/HT2) in rice flour and muesli by LC-MS/MS.

Materials and Methods: Spiked samples of rice flour and muesli, examples of so-called easy and difficult matrices, respectively, were extracted with a mixture of acetonitrile-methanol-water (25/25/50 v/v/v) in an horizontal shaker. Extracts were centrifuged and then diluted with PBS and a volume containing mycotoxins of 1/10 gram equivalents was cleaned up by the multianalyte-IAC. Purified extracts were thereafter measured by a common LC-MS/MS apparatus.

Discussion and Conclusion: For the user it is most notable that multianalyte immunoaffinity separation of regulated mycotoxins together in one step is available at price level of usual one analyte IAC separation. Recoveries found lie within 70-100% of each spiked mycotoxin species in both examples cited. Limits of European authorities are met (EC No. 1881/2006) by the working range of the combined IAC-LC-MS/MS tool. Thus, the analysis of 11 mycotoxins takes just as much time as the analysis of only one mycotoxin. The tool offers even less mass interference and contamination in tandem mass spectrometry than often encountered in normal SPE on adsorber basis or direct injection without any clean up.

Keywords: B-TeZ IAC Combi AOZDFTM, Immunoaffinity column, LC-MS/MS analysis

Reference: DLG, in preparation

P21

PCR-based techniques for aflatoxigenic fungi are alternative ways for aflatoxin risk assessment in developing countries

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Aflatoxins (AFs) are the major mycotoxins that can contaminate crops in areas with hot climate. AFs contamination of crops is a serious issue worldwide because contaminated crops impact quality, marketing and may affect the health of humans and animals that consume the product. In developing countries there is little regulation of contamination so the population may be at risk. To date, we do not know how to detoxify materials contaminated by AFs in ways that retain their edibility. Our safety, therefore, relies on our ability to detect, quantify and avoid this risk. Existing detection methods of the aflatoxigenic fungi are time consuming, labour-intensive, costly, require mycological expertise and facilities. Therefore, it appears necessary to develop new strategies able to detect and quantify AF-producing fungi everywhere. The fast, simple to perform and interpret, and inexpensive and sensitive methodologies for these species detection and quantification such as PCR-based techniques are an attractive approach for AFs risk assessment. The major problem in PCR detection and quantification of AF-producing fungi is due to the fact that AFs are multi-ring structures and therefore require a sequence of structural genes for their biosynthesis. That is why there is no specific PCR marker for any one of the four biologically produced AFs. Unfortunately, the structural genes presently in use for PCR detection of aflatoxigenic fungi are also involved in the synthesis of other fungal toxins such as sterigmatocystin and therefore lack absolute specificity for AF-producing fungi. In addition, the genomic presence of several structural genes involved in AF biosynthesis does not guarantee the production of AF by all isolates of *Aspergillus flavus* and *A. parasiticus* (Levin, 2012). A lot of work should be done by using bioinformatics and different fingerprinting tools to find markers and to design specific primer pairs for different AFs types and high AFs producers.

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P22

Mycotoxin-soil interactions under plastic covers

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Plastic covers are used for white asparagus cultivation, because they provide higher soil temperatures and better moisture conservation, resulting in an early harvest and better crop yield (1). A frequent asparagus disease is the infestation with *Fusarium* sp., with the following synthesis of mycotoxins (2). The aim of this study was to investigate mycotoxin-soil interactions for Fumonisin B1 (FB1) and Zearalenone (ZEN) in covered (CS) and bare soils (BS).

Soil samples (silty loam soil) from white (CS) and green (BS) asparagus crops were collected at the experimental field in Schifferstadt. Depths: 0-5, 15-35, 35-45 cm. Samples were characterized by its total C and N content, humidity and temperature. Adsorption isotherms (0-5 cm depth, range 0.25 – 10 ug/g) were performed according to the EPA guideline 560/6-82-003. Adsorption to soil and Koc were used to characterize the mycotoxin mobility. Soil extracts were analysed by LC-HRMS.

Soil properties can be altered when plastic covers are used: The high temperatures observed in CS (>30°C, 0-5 cm) resulted in a low N_{tot} (0.132 ± 0.014), indicative of a low microbiological activity due to reduced water content (6.67 ± 1.10 %). This may lead to a dissimilar mycotoxin-soil interaction for FB1 and ZEN (Table 1). Poor adsorption rate and low Koc is related to a higher mobility of the mycotoxin in soil with following consequences: i) higher leaching potential to ground water and ii) better availability for plant uptake. But, iii) mobile mycotoxins in soil may be easily degraded by microorganisms. In further investigations the soil-plant mycobiome and interactions between secondary plant compounds and active mycotoxin biosynthesis will be analysed.

Table 1: Adsorption rate and Koc values for FB1 and ZEN in covered and bare soils

Conc. (µg/g)	Covered soil				Non covered soil			
	FB1		ZEN		FB1		ZEN	
	% Adsorption	Koc	% Adsorption	Koc	% Adsorption	Koc	% Adsorption	Koc
0.25	86 ± 6	1.78 ± 0.21	83 ± 4	1.21 ± 0.46	90 ± 4	2.51 ± 0.87	88 ± 9	1.11 ± 0.01
2.50	51 ± 2	0.23 ± 0.02	82 ± 5	1.14 ± 0.34	65 ± 3	0.33 ± 0.16	86 ± 8	0.99 ± 0.11
10.00	37 ± 7	0.14 ± 0.04	83 ± 1	1.09 ± 0.04	29 ± 9	0.10 ± 0.04	88 ± 2	1.61 ± 0.10

(1) Ham, J.M.; Kluitenberg, G.J. & Lamont, W.J. (1993) J. Amer. Soc. Hort. Sci, 118, 188-193.

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P23

***Fusarium graminearum* is able to synthesize ethylene and to downregulate ethylene production**

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The plant hormone ethylene is produced from methionine via 1-aminocyclopropane-carboxylic acid (ACC) by ACC synthases and ACC oxidases. Ethylene is produced after wounding, flower senescence, leaf abscission and fruit ripening but also during pathogen infection. Using GC-MS headspace analysis we observed that *Fusarium graminearum* is able to produce ethylene *in vitro* on methionine supplemented medium. It has been published that *Fusarium graminearum* can take advantage of ethylene signalling for increasing virulence (Chen X, Steed A, Travella S, Keller B, Nicholson P (2009) *Fusarium graminearum* exploits ethylene signalling to colonize dicotyledonous and monocotyledonous plants. *New Phytol.* 182: 975-83.). Ethylene insensitive transgenic wheat (with silenced *EIN2*) showed increased *Fusarium* resistance and reduced mycotoxin contamination. Yet, the role of ethylene is controversial, it is generally associated with the resistance response against fungi. Interestingly, the genome of *Fusarium graminearum* also contains two predicted ACC-deaminase candidate genes, with similarity to a *Trichoderma* ACC deaminase. Downregulation of the ethylene response using ACC deaminases is a strategy frequently employed by endophytic bacteria. We expressed the *Fusarium* candidate ACC deaminases in *E. coli* and could confirm that one has high activity. We also started to disrupt all the candidate genes in *Fusarium* to test their role during plant infection.

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P24

The toxigenic ability of selected isolates of *Fusarium poae* and *Fusarium sporotrichioides*

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The aim of the study was to identify and estimate the occurrence of fungi and its mycotoxins in the selected cultivars of oat grown in organic production after artificial infection of macro- and microconidial *Fusarium poae* isolates. The material were kernels of 3 oat cultivars: Deresz, Siwek and Bingo collected in 2010. Petri dish test was used in order to isolate the fungi. After the mycological analysis for further studies 15 fungi isolates of *Fusarium poae* and *Fusarium sporotrichioides* were chosen. The concentration of mycotoxins were evaluated by ELISA method (R-Biopharm) according to the producer manuals. The test results were defined on Tecan Sunrise reader and analyzed on Magellen software.

Mycological grains analysis of all the oat cultivars showed similar isolated fungi composition and the greatest species diversity occurred for the cv Deresz. The most numerous fungi were from the genus *Alternaria*, *Penicillium* and *Fusarium*. In all tested oat cultivars *A. alternata* was accounted for over 1/3 of the total number of isolates. From 8 isolates identified as *F. poae* by mycological analysis 5 confirmed the accuracy of the identification by PCR technique and 2 isolates were specified as *F. culmorum*. One of the tested isolates identified previously as *F. poae* were not determined to any of the tested species. For 6 isolates identified as *F. sporotrichioides* the correctness of the identification were confirmed for 3 of them, 1 isolate was identified as *F. poae* and 1 as *F. culmorum*. One of the tested *F. sporotrichioides* isolates was not determined to any of the tested species.

In this study all isolates of *F. poae* proved to be producers of DON from 10.47 mg/kg to 96.02 mg/kg and T-2 from 14.38 mg/kg to 737.38 mg/kg. Only one *F. poae* isolate produced ZEA in the amount of 0.39 mg/kg. All tested *F. sporotrichioides* isolates produced DON from 13.69 mg/kg to 37.91 mg/kg and T- 2 from 6.46 mg/kg to 737.38 mg/kg. None of the tested isolates of *F. sporotrichioides* did not produced ZEA.

P25

Sequencing, physical organization and kinetic expression of the patulin biosynthetic gene cluster from *Penicillium expansum*

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Patulin is a polyketide-derived mycotoxin produced by numerous filamentous fungi. Among them, *Penicillium expansum* is by far the most worrisome species. This fungus is a destructive phytopathogen capable of growing on fruits and provokes the blue mold decay of apples. While growing in fruits, the fungus produces significant amounts of patulin. This mycotoxin biosynthetic pathway is chemically well characterized, but its genetic bases remain largely unknown with only few characterized genes in some less economic relevant species. A better understanding of mechanisms leading to patulin production in *P. expansum* will help defining strategies to reduce its occurrence in apple based products. The patulin cluster sequence in *P. expansum* constitutes a key step. It will allow the elucidation of each gene role, but also the study of the mechanisms that regulate patulin production in apple. Therefore, our study consisted on the identification and positional organization of the patulin gene cluster in *P. expansum* strain NRRL 35695. Several amplification reactions were performed with degenerative primers designed based on sequences from the orthologous genes available in other species. A Genome Walking approach was also used in order to sequence the remaining adjacent genes of the cluster. RACE-PCR was also performed from mRNAs in order to determine the start and stop codons of the coding sequences. The patulin gene cluster in *P. expansum* contains 15 genes as recently described in *A. clavatus*. However, the order and orientation of genes differ significantly in comparison with *A. clavatus*. This work was completed by studying the expression kinetics of all the genes in the patulin cluster under patulin permissive and restrictive conditions. The obtained results display a significant positive link between gene expressions and patulin production, confirming the potential involvement of these genes in patulin biosynthesis in this fungus.

Key words: Apple, Gene cluster, mycotoxin, patulin, *Penicillium expansum*.

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P26

Influence of a serin peptidase on the regulation of the amount of ochratoxin A produced by *Penicillium nordicum*

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Penicillium nordicum is a fungal species which usually produces consistently high amounts of ochratoxin A. The biosynthesis of ochratoxin A is regulated by various environmental conditions like for example the growth substrate. However also on a given medium the ochratoxin A production kinetics is not constant over time. Depending on the type of medium the maximum level of ochratoxin A produced is not stable but rather a permanent oscillation can be observed. This demonstrates two opposite mechanisms: biosynthesis of ochratoxin A and a subsequent degradation or derivatization of the molecule. The gene cluster responsible for the biosynthesis of ochratoxin A has been described and consists among others of a polyketide synthase gene (otapksPN) and a non-ribosomal peptide synthase gene (otanpsPN). Interestingly in the vicinity of this cluster also a gene for a serine peptidase (aspPN) can be found. Ochratoxin itself consists of a dihydroisocoumarin polyketide part which is coupled via a peptide bridge to the amino acid phenylalanine. So it contains a peptide bond which can be cleaved by a proteolytic activity. Interestingly the expression of the aspPN gene is different of that of the otapksPN gene and partly coincides with the degradation of ochratoxin A on the respective medium. A strain transformed with a plasmid carrying a part of the protease gene in order to inactivate the gene produced much more ochratoxin A than the wild type. In fact this strain showed a much lower proteolytic activity compared to the wild type. On the other hand a strain transformed with a plasmid containing the whole gene including all transcription signals produced less ochratoxin A than the wild type. These results indicate that the aspPN, a serine protease gene located directly beside the ochratoxin A cluster, is involved in the regulation of the amount of ochratoxin A produced by *P. nordicum*.

P27

Comparative analysis of 39 isolates of *Penicillium verrucosum* reveals significant intraspecific variability

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Penicillium verrucosum is described to produce the mycotoxins ochratoxin A and citrinin, which are mainly nephrotoxic and hepatotoxic. For ochratoxin A, which is rated as class II carcinogen, regulatory limits have been set in several countries. Mycotoxin biosynthesis could be influenced by different environmental growth factors like temperature, water activity, pH, substrate composition, light of visible wavelength and preservatives; in general factors which impose stress to the fungus. *P. verrucosum* could be isolated from habitats which could impose hyperosmotic or oxidative stress to the fungus like fermented meats, olives and cereals. To compete on these unfavourable growth conditions this species has developed distinct adaptation mechanisms, which lead to completely different adapted spore generations resulting in various subtypes of *P. verrucosum* exhibiting quite different optima for growth and mycotoxin biosynthesis. To investigate this proposed intraspecific variability we analysed 39 isolates of *P. verrucosum* on yeast extract sucrose and malt extract agar. We could show that there exists a great intraspecific variability between the different isolates of *P. verrucosum* on level of secondary metabolism and to a lesser extend in their growth rate. Keeping these results in mind, it should be reconsidered if it is valid to use the outcome of growth and mycotoxin biosynthesis experiments at different growth parameters by using only single strains as representatives for the whole species.

P28

Identification of putative mycotoxin gene clusters in *Claviceps purpurea*

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The phytopathogenic ascomycete *Claviceps purpurea* is an important food contaminant as it infects a broad range of grasses including economically important cereal crop plants. The best characterized secondary metabolites of *C. purpurea* are the toxic ergot alkaloids produced in the sclerotia, the survival structure of the fungus¹. Apart from that, little is known about the secondary metabolism of *C. purpurea* and not all toxic substances going along with the food contamination with *Claviceps* are known yet.

The availability of the *C. purpurea* genome sequence allowed a bioinformatical screening approach for typical secondary metabolite key enzymes. The presence of 9 polyketide synthases (PKSs) and 18 nonribosomal peptide synthetases (NRPSs)² shows the great potential of *C. purpurea* for producing secondary metabolites. Most of the NRPSs and PKSs genes are not expressed under the tested standard laboratory conditions but the overexpression of a cluster-specific transcription factor or a global regulator like Velvet could be an efficient way to activate silent gene clusters.

By overexpressing the cluster-specific transcription factor we were able to activate a cluster of genes with high homology to epipolythiodioxopiperazine (ETP) gene clusters in *Leptosphaeria maculans* (sirodesmin) and *Aspergillus fumigatus* (gliotoxin). Mass spectrometry analyses show different peak profiles for the overexpression mutants in comparison to the wild type and further product analyses will show if *C. purpurea* is able to produce a so far unknown secondary metabolite.

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2: Schardl CL, Young CA, Hesse U, Amyotte SG, Andreeva K et al (2013) Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the clavicipitaceae reveals dynamics of alkaloid loci. PLoS Genet 9(2)

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P29

Catechol formation, a novel aspect of sterigmatocystin metabolism

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The mycotoxin sterigmatocystin (STC) has an aflatoxin-like structure including a furofuran ring system. Like aflatoxin B1, STC is a liver carcinogen in rodents and forms DNA adducts after metabolic activation to an epoxide at the furofuran ring. In incubations of STC with various human cytochrome P450 (CYP) isoforms, the formation of three oxygenated STC metabolites has recently been reported, two of which were believed to arise through the epoxidation pathway (Cabaret et al., *Chem. Res. Toxicol.* 23, 1673, 2010). However, discrepancies were noted between the assigned structures and the mass spectra (Kroll, *Chem. Res. Toxicol.* 24, 1339, 2011).

We have reinvestigated the CYP-mediated metabolism of STC by using microsomes from human and rat liver and LC-MS/MS analysis. Moreover, the epoxide of STC, its hydrolysis product STC-dihydrodiol, and the catechol 9-hydroxy-STC were chemically synthesized as reference materials. The hydroquinone 11-hydroxy-STC was obtained as reference compound by microsomal demethylation of 11-methoxy-STC.

The ethyl acetate extracts of microsomal incubations of STC contained three monooxygenated and three dioxygenated metabolites. None of them cochromatographed or had identical mass spectra with synthetic STC-epoxide or STC-dihydrodiol. In contrast, one major and one minor metabolite were clearly identified as 9-hydroxy-STC and 11-hydroxy-STC, respectively, by comparison of their LC retention times and mass spectra with those of the authentic reference compounds. Another major product was tentatively assigned the structure of 12a-hydroxy-STC, arising from hydroxylation at the bridging C-atom of the furofuran ring. Preliminary evidence suggests that the three dioxygenated metabolites are formed by combined hydroxylation at C-9, 11, and 12a.

In summary, our study has shown for the first time that CYP-mediated metabolism of STC comprises extensive hydroxylation at aromatic and aliphatic positions with a catechol as the major metabolite, whereas products of the epoxidation pathway were not observed in the microsomal incubations.

P30

Conjugation of *Alternaria* toxins in tobacco suspension cells

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Mycotoxins produced by various *Alternaria* species frequently contaminate food items such as vegetables and cereals. The major *Alternaria* toxins alternariol (AOH) and its 9-O-methylether (AME) are benzo[a]pyrones with three and two hydroxyl groups, respectively. In mammalian cells, AOH and AME are extensively conjugated with glucuronic acid and sulfate. If conjugation of the hydroxyl groups also occurred in plants, so-called "masked" mycotoxins would be formed, which escape routine methods of food analysis.

In order to investigate this hypothesis, AOH and AME were incubated with tobacco BY-2 cells. Incubations were conducted with 50 μ M toxin during the exponential growth phase for four days at 26°C in the dark with gentle shaking. Cells were collected by filtration, freeze-dried, grounded in a mortar, and extracted with water/methanol and methanol/dichloromethane. Analysis of the combined cell extract using LC-MS/MS showed that only very small amounts of non-conjugated AOH and AME were present. Five polar metabolites of AOH and three of AME were detected as major products, which, according to their MS/MS and UV spectra, were tentatively identified as conjugates with one or two hexoses and malonylhexoses. The exact chemical structures will be clarified in future studies. The incubation medium was directly extracted with methanol/dichloromethane and contained no free toxins and only small amounts of conjugates.

Our results show for the first time that AOH and AME are completely taken up and extensively conjugated by plant cells. Thus, *Alternaria* toxins of the benzo[a]pyrone type are prone to form masked mycotoxins. If these are not taken into account, the total exposure to *Alternaria* toxins may be underestimated.

P31

Optimization and characterization of polygalacturonase enzyme produced by gamma irradiated *Penicillium citrinum*

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The activity of polygalacturonase (PGase) produced by *Penicillium citrinum* was studied in solid state fermentation (SSF) using sugar beet pulp as substrate. A full factorial design was used to study the effect of 5 variables (yeast extract, pH, inoculum size, incubation period, and incubation temperature) on polygalacturonase production.

Maximum polygalacturonase (PGase) production (132 U/g dry fermented substrate (U/gdfs) was obtained in 8 days at 30°C and pH 5.5 with yeast extract as the best nitrogen source (1.2%) inoculated with 1 ml of spore suspension (1.8×10⁵ spores/ml). Using the optimized conditions of factorial design, maximum PGase production has been obtained by using 0.7 kGy of gamma radiation with an activity 152.2U/gdfs as compared to the parent strain (unirradiated).

The enzyme was partially purified using 75% ammonium sulphate precipitation, dialysis, and gel filtration chromatography on sephadex G-100. The optimum pH and temperature of the enzyme were found to be 6.0 and 40°C respectively. The enzyme was found to be stable in the pH range 4-8 and showed high stability at temperature range 20-60°C. The effect of metal ions on enzyme activity was investigated. It was found that Mg²⁺ and Zn²⁺ stimulated PGase activity.

P32

Biotransformation of zearalenone by fungi used in food fermentation

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Zearalenone (ZEN), an estrogenic mycotoxin, is produced by *Fusarium* spp., which commonly infest grains. The infected host plant as well as other fungal species can transform ZEN into different conjugated forms (e.g. glucosides or sulfates) in the field. In addition to these naturally occurring ZEN conjugates, fermentation of food using fungi may lead to similar ZEN derivatives. *Aspergillus* and *Rhizopus* are common fungal species that are used for production of fermented soy and cereal products, and that have been described to catalyze ZEN transformation. Because the unfermented cereal and soy raw materials may contain ZEN, masked ZEN derivatives may be formed during food processing.

The objective of this study was to investigate the biotransformation of ZEN by various fungi. For a first qualitative overview seven *Rhizopus* and two *Aspergillus* species including technological relevant strains were screened for ZEN metabolite formation. The metabolite screening was conducted by adding ZEN to liquid cultures of the different fungal species. After three days the media were analyzed for metabolite formation by HPLC-MS/MS. All investigated strains were able to convert ZEN into at least five metabolites accompanied by rapid disappearance of ZEN. α -Zearalenol (α -ZEL), α -ZEL-sulfate, ZEN-14-sulfate, ZEN-O-14- and ZEN-O-16-glucoside were the prevalent conversion products. The formation of α -ZEL-sulfate and ZEN-O-16-glucoside as fungal metabolites is described for the first time.

The present study showed that the investigated *Rhizopus* and *Aspergillus* strains have a high potential for ZEN masking. However, the detected ZEN conjugates are not covered by most routine analyses due to the lack of pure reference standards. Thus, for further studies on fermented food, ZEN conjugate standards are urgently needed. Additional to novel findings potentially affecting the safety of fermented food, our results may enable biosynthetic strategies for obtaining required standard substances.

P33

***Fusarium graminearum* can subvert the synthesis of tryptamine derived defense compounds to produce auxin**

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Indole-3-acetic acid (IAA) is the most important auxin in plants and responsible for a variety of developmental processes like cell division, cell differentiation, phototropism, root gravitropism. It is the major growth hormone and also a negative regulator of resistance to many plant pathogens. In response to *Fusarium graminearum* infection monocot host plants upregulate tryptophan biosynthesis to produce tryptamine (TAM) and derived defense compounds such as hydroxycinnamic acid amides of tryptamine (e.g. coumaroyl-TAM, feruloyl-TAM) which are antifungal and used to reinforce the cell wall. Also the TAM hydroxylation product serotonin is used to form the corresponding conjugates (coumaroyl-serotonin, feruloyl-serotonin, etc.). We found that *Fusarium graminearum* is able to efficiently convert TAM, which is present in high amounts in infected tissue, into IAA (and serotonin into 5-hydroxy-IAA).

The first step of this reaction, conversion of TAM into indole-3-acetaldehyde (unstable intermediate) is catalyzed by amine oxidases. We expressed all seven predicted *Fusarium* copper-amine oxidase genes in *Saccharomyces cerevisiae* and tested them for activity with tryptamine. Three products of the candidate genes showed activity. Deletion of the gene AOX4, which was the most active in yeast, strongly impaired auxin production by the knock-out strain (low amounts of IAA were formed very late). We are currently trying to create triple mutant strains to test whether IAA production can be completely eliminated and whether this has an impact on fungal virulence. Our hypothesis is that *Fusarium graminearum* can divert tryptamine away from the cell wall mediated defence pathway and convert it into a signal increasing plant susceptibility by redirecting resources from defense to plant growth.

P34

Fungal allelopathy suppresses anti-fungivore defence in a mycotoxigenic mould

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Grazing by invertebrate animals is an important factor affecting mycotoxin-based anti-fungivore defence. Animal fungivory has also been shown to influence fungus-fungus interactions, however, the reverse, how fungal competition regulates anti-fungivore properties, e.g. mycotoxin formation, and thereby determines invertebrate success and fungal fitness remains largely unknown. We demonstrate, for a tripartite fungal-insect model system, that habitat-specific release of metabolites by the yeast *Saccharomyces cerevisiae* suppresses morphological and chemical differentiation of the mycotoxigenic mould *Aspergillus nidulans*. This phenotypic shift leads to consistently reduced capacities to harm fungivorous larvae of the fruit fly *Drosophila melanogaster* and impairment of the inducibility of mycotoxin-related genes involved in resistance to insect grazing. Our study supports the notion that heterogeneity in the outcome of fungus-fungivore interactions is linked to variation in inducible mycotoxin-based defence.

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P35

The role of fumonisins in interaction of *Fusarium verticillioides* with maize plants and with *Fusarium graminearum*

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Fumonisins are secondary metabolites produced by *Fusarium verticillioides*. They are mycotoxins which have toxic effects on humans and livestock. *F. verticillioides* is considered as a major causal agent of pink ear rot disease in maize crop worldwide. It affects maize production not only by reducing the yield, but the infection also deteriorates maize kernels quality due to contamination with fumonisins. *F. verticillioides* is an endophytic fungus grows within host plants tissues and the infection can be asymptomatic or symptomatic.

To elucidate the role of fumonisins produced by the fungus in the disease development on maize cobs, a wild type strain of *F. verticillioides* producing fumonisins and a mutant strain with fumonisins biosynthesis disruption were used for inoculation of maize variety Gaspe Flint in a greenhouse. An additional maize pathogen *Fusarium graminearum* was inoculated subsequently into the same plants.

The results showed that both strains of *F. verticillioides*, the wild type strain and the mutant, were asymptomatic or developed very mild symptoms on maize cobs. Subsequent infection with *F. graminearum* increased disease severity in both strains of *F. verticillioides*. The quantification of *F. verticillioides* biomass via real-time PCR revealed no significant differences in the growth of both strains individually on kernels of living plants. The subsequent infection with *F. graminearum* caused a remarkable increase of *F. verticillioides* biomass, which was the same for the fumonisins-deficient mutant and the wild type strain that produces detectable amounts of fumonisin B1 in infected plants.

The results implies that fumonisin is not a virulence factor in *F. verticillioides* infection of maize variety Gaspe Flint and that consequent infection with *F. graminearum* facilitates colonization of maize cobs with *F. verticillioides*, probably by interfering with plant defence responses that control the spread of *F. verticillioides* in the tissues.

P36

Role of mycotoxins in fungal pathogen populations

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The ability of fungal plant pathogens to cope with toxic compounds is an important element of competition in the environment. The interactions between producers and non-producers influence both population equilibria and infectious processes. Therefore, even the non-producing filamentous fungi usually preserve a number of adaptations related to the adaptation to the mycotoxin presence in the environment. Traditionally, the toxic molecules have been viewed solely as harmful compounds. While one of the main coping strategies is indeed detoxification or efflux of mycotoxin molecules, a widespread but less toxic compound can also serve as a potential signal enabling adaptive reaction. We investigated the potential adaptation of toxins as molecular signals in the interactions between isolates differing in susceptibility to mycotoxins and toxigenic potential. We examined effects of toxins on morphology, growth patterns and gene expression after stimulation in both mycotoxin producing and non-producer isolates of divergent *Fusarium* species.

We observed a distinct “producer effect” (producing species being more resistant, relic resistance in species that lost the biosynthetic capacity), as well as inferred a possible explanation for chemotype divergences due to adaptation of some toxins, as signalling molecules. In particular deoxynivalenol, a mycotoxin commonly produced by the widespread and aggressive cereal pathogen *Fusarium graminearum* shows distinct patterns of influence on both morphology and gene expression of both producer and non-producer species, above and beyond the (potential) toxicity. Also, the non-producing *Fusarium verticillioides*/*Fusarium proliferatum* isolates show a direct response to deoxynivalenol. The toxin in the medium has strong effects on the mycelial growth rate, number of viable fungal cells in medium and early gene expression, in particular of the genes related to biosynthesis and transport of fumonisins.

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P37

Proteome studies of emmer and naked barley after *Fusarium graminearum* infection during grain development

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Fusarium head blight (FHB) is a serious disease of small grain cereals, causing significant losses of crop yield due to the accumulation of mycotoxins, such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA).

With this study we investigated the impact of *F. graminearum* infection on the grains of emmer (*Triticum dicoccum*) and naked barley (*Hordeum vulgare nudum*), regarding the whole course of grain ripening and from milk ripe to harvest stage. Given that cereal albumins and globulins contain numerous proteins with relevant functions such as cell development, metabolism and stress response we expect new information concerning pathogenicity and host response to *F. graminearum*.

The crops were grown in a field trial near Göttingen. For the analysis, ears from each plot were collected at milk ripe, soft dough, hard dough and plant death stage. Proteomic studies were conducted to analyse the effect of *Fusarium* inoculation on emmer and naked barley grains during the entire grain ripening and infection-period beginning with early grain development (milk ripe) until plant death.

Different proteins have been induced depending on the grain ripening stages and the cereal genotype. In both genotypes chitinase played an important role. Furthermore, proteins related to carbohydrate metabolism and photosynthesis showed higher abundance in inoculated grains at earlier ripening stages. In naked barley several protease inhibitors as well as antimicrobial proteins such as thaumatin-like proteins were identified, whereas in emmer a spermidin synthase and xylanase inhibitor protein were detected.

These results demonstrate that different mechanisms are initiated by *Fusarium* infection, depending on the genotype and the respective development stage.

P38

Genotoxicity of 4-hydroxy-alternariol and its methyl ether in comparison to the parent substances alternariol and alternariol monomethyl ether

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Alternaria toxins belong to the so-called emerging mycotoxins with respect to their frequency of occurrence and gaining toxicological concern. Alternariol and its methyl ether have been reported to represent common substrates for cytochrome P450 enzymes in cells, generating hydroxylated metabolites. But so far, the impact of phase I metabolism on the DNA damaging properties of the parent substances have never been investigated before. In the present study, the metabolites 4-hydroxy alternariol and 4-hydroxy alternariol monomethyl ether were synthesized and characterized respectively. Genotoxic properties and the mode of action were investigated in human oesophageal cells in comparison to the parent compounds. Although both metabolites were bearing a newly formed catecholic structure, no onset of oxidative stress, nor enhanced levels of oxidative DNA damage were observed. These data are in line with the lower stabilizing effect of the phase I metabolites on the topoisomerase II-DNA-intermediates compared to the respective parent compounds. We conclude from this study that the metabolization of alternariol and alternariol monomethyl ether by the phase I enzyme family cytochrome P450 is a step towards detoxification heading in the direction of phase II conjugation and subsequent elimination, which is currently under investigation.

P39

Effects of *Fusarium* mycotoxins on the blood-brain barrier

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Fungi of the *Fusarium* genus produce secondary metabolites which exhibit toxic properties. Although neurotoxic effects of these mycotoxins have been discovered, a systematic study on the effects of *Fusarium* mycotoxins on the blood-brain barrier is still missing. To induce direct effects in neurons or other cells within the brain, mycotoxins would require to penetrate or weaken the blood-brain barrier.

In the present study results of an *in vitro* cell culture model are presented. Porcine brain capillary endothelial cells (PBCEC) were seeded on Transwell[®] inserts. PBCEC form a monolayer as well as strong tight junctions. The tightness of the barrier was ensured by transepithelial electrical resistances (TEER) of $>600 \Omega \cdot \text{cm}^2$ using a CellZcope[®] impedance spectroscopy. The obtained values are much higher and closer to the barrier integrity *in vivo* compared to commercial brain capillary endothelial cell lines. Before applying the test compounds in transport studies, they were tested for their effects on cellular viability using the Cell Counting Kit-8 (CCK-8).

Fumonisin (FB₁, HFB₁), moniliformin (MON), trichothecenes (DON, 3-Ac-DON), zearalenone (ZEN) and its metabolite α -zearalenol (α -ZEL) were chosen as test compounds to cover a wide range of polarity and molecular size.

The data show that DON, 3-Ac-DON and FB₁ disturb the blood-brain barrier integrity by decreasing TEER or electrical capacitance (C_d), when applied in 10 μM concentration in the apical compartment. Furthermore, it was possible to detect and quantify phase I and II metabolites of ZEN, α -ZEL.

P40

Inhibition of human and bacterial topoisomerase IIA by perylene quinone-type *Alternaria* toxins

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Alternaria species are fungi found as contaminants on wheat, sorghum, barley, as well as in various nuts, fruits and vegetables. In the 1960s an apparent correlation between the consumption of food contaminated with *Alternaria* mycotoxins and an increased incidence of esophageal cancer was reported in the Linxian region in China. Data of the toxicity of *Alternaria* toxins is still scarce (EFSA (2011) Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. EFSA Journal 9(11):2407). One structural class of the more than 70 secondary metabolites produced by *Alternaria* spp. are the perylene quinone-type mycotoxins. A representative of this class is altertoxin II (ATX II), which has previously been identified as a genotoxic impact compound of complex *Alternaria* toxin mixtures. However, the underlying mechanism of action has not been identified yet. Recently ATX II has been found to affect the activity of topoisomerase II α (top II α). Topoisomerases are essential enzymes in the regulation of DNA topology in eukaryotes and prokaryotes, involved in fundamental mechanisms of cellular proliferation. Top II α belongs to the type IIA family of topoisomerases, which insert transient double strand breaks into their substrate DNA. Targeted by several anticancer agents, the modulation of type IIA topoisomerases can lead to DNA double strand breaks and therefore interfere with the genetic stability. The bacterial analogon of mammalian top II α is the type IIA topoisomerase gyrase, which is the target enzyme of diverse classes of antibiotics since the 1960s.

In recent studies we demonstrated that human top II α is a target of four perylene quinones, isolated from *A. alternata*, ATX II, altertoxin I, alterperyleneol and stemphytoxin III. In addition to the inhibition of mammalian top II α , these four *Alternaria* toxins also showed modulatory effects on *E. coli* gyrase, thus exerting potential antibacterial activity.

P41

Examination of the effect of deoxynivalenol (DON) and de-epoxy-deoxynivalenol (DOM-1) on porcine intestinal and immune parameters

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Deoxynivalenol (DON), a contaminant of feed, is known to affect intestinal and immune parameters, particularly in pigs. The intestinal bacterium, Genus *novus* species *novus* BBSH 797, is capable of transforming DON to the less toxic metabolite de-epoxy-deoxynivalenol (DOM-1) and therefore qualifies as a valuable feed additive. Intestinal epithelial cells present the primary target of DON following the ingestion of contaminated feed. DON disrupts the intestinal epithelial barrier, allowing paracellular passage of xenobiotics, harmful molecules, and microorganisms. In addition, DON is known for its inhibitory effect on cellular protein synthesis and its induction of apoptosis. Thus, tissues with high protein turnover or quickly proliferating cells, such as immune cells, like peripheral blood mononuclear cells (PBMCs), are highly sensitive to DON. Therefore, the effect of DON and DOM-1 on the barrier function of differentiated porcine intestinal epithelial cells (IPEC-J2) and the proliferation of PBMCs was measured. IPEC-J2 cells were differentiated in Transwell® membrane inserts and treated with DON or DOM-1 [both: 0.5 – 100 µM]. Transepithelial electrical resistance (TEER, daily) and cytotoxicity (Neutral Red) was recorded over 72 h. DON significantly decreased TEER between 10 – 100 µM (24 h (IC₅₀: 25.91 µM) and 48 h (IC₅₀: 19.53 µM)) and 5 – 100 µM (72 h (IC₅₀: 14.08 µM)). Viability was significantly decreased at 50 and 100 µM DON (IC₅₀: 48.16 µM). In contrast, DOM-1 did not affect permeability or viability of IPEC-2 cells. In parallel, immunorelevant effects of DON and DOM-1 were studied by examining proliferation (BrdU) of PBMCs isolated from porcine blood. DON decreased proliferation at concentrations of and exceeding 250 ng/mL [0.84 µM], whereas DOM-1 was over 400 times less anti-proliferative than DON. Data confirm the detoxification mechanism of Genus *novus* species *novus* BBSH 797 and support its use as a DON detoxifying feed additive.

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The combined application of a practical-relevant DON-concentration and LPS leads to a synergistic up-regulation of IL-8 in MoDCs

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The human and their immune system are confronted with mould-contaminated food and/or mould-contaminated air in rooms. This results in health stress and unspecific disease symptoms. Other studies supply evidence for the connection between allergy and the exposure to mould. The cause of allergy-mediated effects due to mould is still unknown. Deoxynivalenol is due to its frequent occurrence in toxically relevant concentration of high importance. The exposure to this toxin is a permanent health risk for both human and farm animals because DON can not significantly removed during standard milling and processing procedures. However, the direct effect on immunity or haematology is poorly defined because most investigations could not separate the effect of DON-contaminated feed intake. Due to the widespread distribution of DON after rapid absorption it is not surprising that DON is known to affect the immune system. The immune system of the organism have one important function to defend against invade unknown substances/organisms. This study shows for the first time a synergistic effect of both – low physiological DON-doses in combination with low LPS-doses with the focus on the IL-8-expression on protein- and RNA-level. Both doses were found *in vivo*. IL-8 together with other anorectic cytokines like IL-1 β can affect the food intake and anorexia. We could also show that a calcium-response is not involved in the increased IL-8 production after acute DON-stimulation with high or low concentrations.

P43

Deoxynivalenol in chicken feed affects the welfare parameters

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Poultry feeds are frequently contaminated with deoxynivalenol (DON) mycotoxin. The impacts of DON contamination of poultry feeds on a wide range of significant health and immunological parameters were intensively studied. However, the effects of DON contamination of broiler diet on the welfare related parameters are poorly investigated, only a few reports showed that DON increased the stress index (heterophil to lymphocyte ratio, H/L ratio) in broilers (Ghareeb et al.2012).

In the present study, the effects of contamination of broiler diet with 10 mg DON/kg feed on plasma corticosterone and heterophil to lymphocyte (H/L) ratio, an index for fear response and growth performance of broiler chickens were studied. In addition, the effect of MycofixH Select (Biomin GmbH, Herzogenburg, Austria) either alone or in combination with DON contamination on these welfare parameters was also evaluated.

The results showed that DON feeding affected the welfare related parameters of broiler chickens. The feeding of DON contaminated diet resulted in an elevation of plasma corticosterone, higher H/L ratio and increased the fear levels as indicated by longer duration of tonic immobility reaction. Furthermore, DON reduced the body weight and body weight gain during starter and grower phases.

In conclusion, the results showed that the DON feeding increased the underlying physiological stress responses of broilers and resulted in a reduction in the welfare status as indicated by higher plasma corticosterone, higher H/L ratio and higher fearfulness. Additionally, feeding a commercial antidote MycofixH Select (Biomin GmbH, Herzogenburg, Austria) was effective in reducing the adverse effects of DON on the bird's welfare.

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P44

Large scale synthesis and characterization of deoxynivalenol-3-sulfate, a major chicken metabolite

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Deoxynivalenol (DON) and its derivatives are very common and widespread contaminants in food and feed. They are predominantly produced by different *Fusarium* species and can afflict human and animal health after uptake. Beside the occurrence of their masked forms in plants many parent toxins also undergo phase-II-metabolism in living organisms leading to their corresponding glucuronides as well as to their sulfates. In contrast to the occurring glucuronides, which are already accessible by chemical synthesis, little is known about possible occurring trichothecene sulfates. Since DON-3-sulfate (Wan, D., et. al., (2014) Metabolism, distribution, and excretion of deoxynivalenol with combined techniques of radiotracing, high-performance liquid chromatography ion trap time-of-flight mass spectrometry, and online radiometric detection, J. Agric. Food Chem. 62, 288-296) was recently identified as the main chicken metabolite, we aimed for a large scale synthesis allowing full NMR characterization of this new metabolite. For this reason 160 mg DON were used as starting material and the sulfation was done utilizing a recently reported sulfation reagent (Ingram, L. J., et. al., (2009) O- and N-Sulfations of Carbohydrates Using Sulfuryl Imidazolium Salts, J. Org. Chem. 74, 6479-6485). The synthesis of the desired protected intermediate was carried out via sulfation reagent and 1,2-dimethylimidazole as base, followed by the cleavage of the remaining sulfate protective group via Zn-dust/ammonium formate. Finally the deprotected target compound was subjected to column chromatography with dichloromethane/methanol/ammonia to obtain the desired metabolite (Fig. 1) as the corresponding ammonia salt. After final lyophilization 114,4 mg of the desired target compound were obtained and characterized using NMR spectroscopy.

The isolated and characterized new metabolite will serve as valuable reference material for numerous further studies within the field of trichothecene-sulfates.

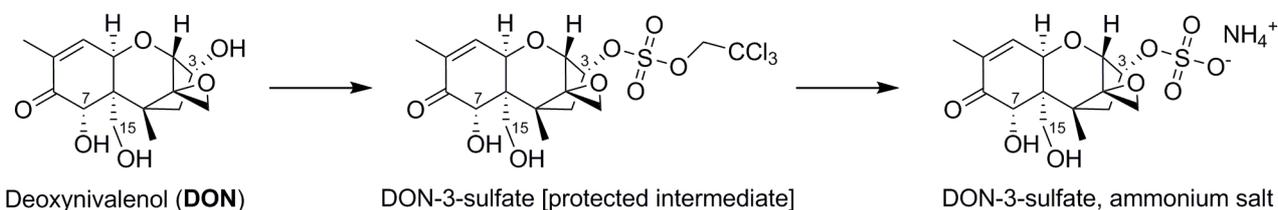


Figure 1: Sulfation of DON and deprotection towards DON-3-sulfate

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P45

Individual profiles of ochratoxin A and citrinin and their metabolites in human blood and urine

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Co-occurrence of ochratoxin A (OTA) and citrinin (CIT) in food commodities may result in combined human exposure to these nephrotoxic mycotoxins. Several studies have analyzed OTA in human blood and urine, whilst biomonitoring for CIT is still in its infancy. We now analyzed OTA and CIT as well as their metabolites ochratoxin alpha (OT α) and dihydrocitrinone (HO-CIT) in blood and urine to obtain individual profiles over time and to gain more insight into the variability of biomarkers for both mycotoxins.

Blood plasma and first morning urines were collected from a 30y old volunteer (A) over a period of 7 weeks and a 60y old volunteer (B) on 7 days in 2 weeks. OTA and OT α were determined by a validated method with HPLC-FLD and suitable low LODs for OTA and OT α in plasma and urine [Muñoz K et al. 2010, J Chrom B 878: 2623–2629]. CIT biomarker analysis used a novel sensitive method with LC/MS-MS detection and low LODs in both matrices [Blaszkewicz M et al. 2013, Arch Toxicol 87:1087-1094].

The analyte profiles in the first individual in blood and urine showed small fluctuations, and mean concentrations of OTA, CIT and their metabolites were clearly lower than in the second individual over the period of time.

	Plasma mean conc (ng/mL)				Urine mean conc (ng/mL)			
	OTA	OT α	CIT	HO-CIT	OTA	OT α	CIT	HO-CIT
Individual A (7 w)	0.41	0.46	0.27	0.28	0.06	0.06	0.03	0.08
Individual B (7 d)	1.64	0.20	0.47	0.96	0.22	2.22	0.05	0.32

Biomarker levels in plasma of both individuals were considerably higher than those measured in urine (plasma/urine ratio for OTA >6, for CIT \geq 9 and for HO-CIT \geq 3), except for OT α which person B excreted at much higher levels than person A, possibly due to more active detoxification of OTA to OT α . Finally, it can be concluded that interindividual variability for the investigated biomarkers reflects dietary exposure and/or disposition of ingested mycotoxins.

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P46

Establishment of a cost-efficient prescreening method for the impact of mycotoxins on miRNA profiles

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Since the discovery of micro-RNAs (miRNA) in the 1990s, their role in post-transcriptional regulation of genes steadily gained importance. These miRNAs play a crucial role in gene expression by acting as sequence specific down-regulators of already transcribed messenger-RNAs (mRNA). At present, over 1800 distinct miRNA sequences, referred to as miRNA species, have been identified in the human genome. An increasing number of publications demonstrate the impact of miRNAs on the response of cells to xenobiotics, raising the question on the existence of compound- and/or cell-specific miRNA response pattern.

DNA biochip technology represents a very quick and global method to analyse such miRNA expression patterns. However, commercially available microarrays for miRNA profiling are still cost intensive, thus limiting attempts towards comprehensive studies on the impact of test compounds such as mycotoxins on miRNA profiles. Here we present independently designed miRNA microarray chips manufactured by Maskless Microarray Synthesis. On basis of the Sanger miRBase Sequence Data Base (release 19) each of the 1872 human miRNA sequences is represented in features (20-22) adherent on the glass biochip. One of such a feature consists of millions of complementary DNA sequences of one miRNA sequence. The collectivity of these features for all miRNA species constitutes the biochip. First measurements of the effect of the *Alternaria* toxin alternariol on the miRNA pattern in HepG2 liver carcinoma cells indicate among others the up-regulation of miR-371b and members of the miR-548 family. miRNA-548 was previously described as specifically targeting and degrading mRNA, coding for the pro-inflammatory agent IFN- λ 1 (Yongkui Li, Jiajia Xie, Xiupeng Xu, Jun Wang, Fang Ao, Yushun Wan, Ying Zhu (2013) *MicroRNA-548* down-regulates host antiviral response via direct targeting of IFN- λ 1. *Protein Cell* 2013, 4(2): 130–141). These results hint at possible immune suppressive effects of alternariol.

P47

Biomonitoring of ochratoxin A in blood plasma of Bangladeshi students

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Ochratoxin A (OTA), a mycotoxin known for its nephrotoxic, immunotoxic and carcinogenic effects in animals, deserves attention due to its widespread occurrence as food contaminant. Studies in many countries report the presence of OTA in human blood plasma or serum at variable levels [1]. However, no biomonitoring study has been carried out in Bangladesh so far, and also food analysis data are insufficient to assess human OTA exposure.

Therefore, 64 blood samples were collected from healthy adult students (32 female, 32 male) in Bangladesh for biomarker analysis: OTA and its metabolite ochratoxin-alpha (OT α) were determined in plasma samples by a validated method using HPLC-fluorescence analysis after liquid-liquid extraction [2] with a LOD of 0.05 ng/mL.

OTA was detected in all plasma samples (100%) at a range of 0.20 – 6.63 ng/mL, and OT α in 95% of the samples at 0.05 – 0.79 ng/mL. The OTA mean level in plasma of male (0.92 ± 1.09 ng/mL) and female (0.78 ± 1.02) students were not significantly different. Statistical analysis of food consumption data for the participants, provided in a food frequency questionnaire, did not reveal a significant association between OTA level in plasma and their intake of typical staple foods (rice, wheat, maize, lentil chicken meat, milk products).

The dietary intake of OTA (mean 11.7, max 91.4 ng/kg bw/week) calculated on the basis of plasma concentration in Bangladeshi students was lower than the tolerable weekly OTA intake (120 ng/kg bw/week) set by EFSA. Nonetheless, further biomonitoring is recommended in cohorts from other parts of the country that may have higher mycotoxin exposure than the present group.

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P48

Zearalenone and hormones in blood serum of young wild boar sows hunted for in forests and large corn field areas

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The mycotoxin zearalenone (ZEN) is a secondary metabolite of fungi which is produced by certain species of the genus *Fusarium* and can occur in cereals and other plant products. As endocrine disrupting compounds work through receptors and/or hormone steroidogenesis pathways and have the potential to alter rates and concentrations of hormones in blood (progesterone, estradiol, testosterone).

The aim of the study was the evaluation of ZEN and its metabolites content (using HPLC MS/MS) in blood serum of young wild boar sows (50-60 kg body weight) hunted for from November 2011 to January 2013. The animals came from large corn fields areas (2012 n=14; 2013 n=22) and forests (2012 n=12; 2013 n=16) – control area. Additionally hormones were evaluated using IMMULITE 2000 (Siemens) in the laboratory of medical analysis. The results have been presented in the tables below.

Mycotoxins in blood serum		ZEN	ZAN	α ZOL	β ZOL	α ZAL	β ZAL	
Field area	2012	Samples/Positive samples	14/14 (100%)	14/6 (43%)	14/4 (29%)	14/1 (7%)	14/0 (0%)	14/0 (0%)
		Mean	0.27	<0.09	<0.10	nd	nd	nd
	2013	Samples/Positive samples	22/22 (100%)	22/15 (68%)	22/12 (55%)	22/1 (5%)	22/1 (5%)	22/1 (5%)
		Mean	0.635	0.264	0.111	nd	nd	nd
Forest	2012	Samples/Positive samples	12/6 (50%)	12/3 (25%)	12/1 (8%)	12/0 (0%)	12/0 (0%)	12/0 (0%)
		Mean	0.066	<0.09	nd	nd	nd	nd
	2013	Samples/Positive samples	16/16 (100%)	16/8 (50%)	16/9 (56%)	16/4 (25%)	16/3 (19%)	16/1 (6%)
		Mean	1.02	0.17	2.28	0.20	nd	nd

nd – not detected

Hormones in blood serum		FSH [mIU/ml]	LH [mIU/ml]	E2 [pmol/l]	PRL [ng/ml]	PGN [ng/ml]	TEST [ng/dl]	DHEA(s) [mg/dl]
Field area	2012	<0.1	<0.1	59.4	0.59	7.06	63.4	<15
	2013	0.24	0.39	218	0.67	7.83	90.1	<15
Forest	2012	0.12	0.14	178	0.51	10.6	172	15.1
	2013	0.27	0.21	590	0.57	9.34	167	<15

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P49

Kinetic studies of deoxynivalenol (DON) and its metabolites, DON sulfonates (DONS) 1, 2 and 3 with sodium sulfite treated DON contaminated maize in the pig

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DON intoxications might cause serious problems in pig nutrition when critical dietary toxin concentrations are exceeded. As DON contamination of agricultural crops may not be completely prevented, detoxification measures are needed. In a previous experiment it could be demonstrated that wet preservation with sodium sulfite resulted in a significant reduction of the DON concentration in maize (Paulick M, Rempe I, Kersten S, Schatzmayr D, Schwartz-Zimmermann H E, Dänicke S (2014) Effects of increasing concentrations of sodium sulfite on DON and DON sulfonate concentrations of different feed matrices preserved with propionic acid at various moisture contents. In preparation). The preserved material had a characteristic DONS pattern. Therefore the properties of the preserved maize should be tested *in vivo*.

In order to investigate the toxicokinetics and bioavailability of DON and DONS in pigs, different variants of oral administration and i.v. application were tested. The calculation of the area under the curves of the substance concentrations vs. time curves should enable to evaluate the systemic absorption of the individual compounds.

The study was carried out with 16 male castrated pigs with a mean body weight of 39 kg, which were housed in balance cages. For serial blood sampling pigs underwent surgery to be equipped with two permanent intravenous catheters in the external jugular veins. After collecting blood samples were centrifuged to separate plasma and kept frozen until measurement of DON and DONS 1, 2 and 3 with a (U)HPLC-LC-MS/MS method.

The preliminary analysis of measured values showed decreased DON concentrations in plasma of pigs fed with sodium sulfite treated DON diet compared to DON diet without addition. The low DON and high DONS concentrations of sodium sulfite preserved variants were partially confirmed in plasma but no DONS could be detected in plasma so far. Therefore, further investigations are essential.

P50

Detoxification of ochratoxin A by lactic acid bacteria

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Ochratoxin A (OTA) is one of the most important mycotoxins found in food and feed products. It is produced by several species of *Aspergillus* and *Penicillium* that can be found in a wide variety of agricultural products, which makes the presence of OTA in these products common. OTA has been associated with human kidney disease, referred to as Balkan Endemic Nephropathy. IARC has also classified OTA as group 2B (possibly human carcinogenic).

Because biological decontamination of mycotoxins using microorganisms is one of the well-known strategies for the management of mycotoxins in foods, the aim of the present study was to investigate the ability of lactic acid bacteria (LAB) strains to remove OTA from an aqueous medium using different parameters. The concentration of OTA was measured in the supernatant using HPLC.

The strains exhibited different degrees of OTA removal, with two highly effective strains recognized: *Lactobacillus casei* which caused a decrease of OTA by 90.93% and *Lactobacillus gasseri* LA 39 which reduced OTA level by 78.55%. Using a mixture of both strains the level of OTA removal was decreased and reached 31.66%. Results also showed that the removal of OTA depended on different parameters, i.e. LAB strains, concentration of the OTA, the pH value, and the incubation period. It was noticed that removal of OTA was considered a rapid process with more than 60% of OTA removed after five minutes. The stability of the LAB/OTA complex was evaluated by determining the amount of OTA removed by repeated washing, and it was noticed that removal of OTA was to a limited degree reversible, and that *L. casei*/OTA complex exhibited the greatest stability with 86.98% of OTA remaining bound.

It could be concluded that the removal of OTA occurred through binding to the bacterial biomass. However, these results must be supported by in vivo experiments in order to assess the effect of LAB on OTA bioavailability and toxicity.

P51

Aerobic and anaerobic *in vitro* testing of deoxynivalenol-detoxifying feed additives

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Deoxynivalenol (DON), a mycotoxin of the group of trichothecenes produced by fungi of the *Fusarium* genus, occurs in toxicologically relevant concentrations worldwide in cereals. Several DON reducing strategies have been developed. One approach is the biotransformation into non-toxic metabolites by the use of microbes or enzymes applied as feed additives.

Aim of the study was to test the efficacy of 19 commercially available products claiming to detoxify DON *in situ*. Therefore, buffer solutions (pH 7) each containing 0.5% w/v of the agent and 5 mg/L DON were incubated aerobically under shaking as well as anaerobically (under H₂ and cysteine Na₂S exposure) for 72 h at 37 °C. An aerobic DON-degrading bacterium and a lyophilisate of the anaerobic strain BBSH 797, which is able to biotransform the epoxide group of trichothecenes into a diene (Fuchs E, Binder EM, Heidler D, Krska R (2002) Structural characterization of metabolites after the microbial degradation of type A trichothecenes by the bacterial strain BBSH 797. Food Addit. Contam 19:379–386), were used as positive controls. Samples were taken directly after product addition as well as after 1, 3, 24, 48 and 72 h of incubation and analysed by LC-DAD-MS/MS.

Apart from the positive controls, only one of the tested products was able to completely degrade DON into the equimolar concentration of the non-toxic metabolite deepoxy-deoxynivalenol (DOM-1) within 48 h under the applied anaerobic conditions. For all other products tested under aerobic and anaerobic conditions an average DON reduction of $1 \pm 10\%$ after 72 h of incubation with a maximum of 17% for one product (no metabolites detected) was observed.

Our results indicate that only 1 out of 19 commercially available feed additives was sufficiently effective in detoxifying DON under the applied conditions. This demonstrates the necessity of *in vitro* experiments, to critically screen agents claiming mycotoxin detoxification.

P52

Antifungal activity of Indonesian selected brown seaweeds extract against *Aspergillus flavus*

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Brown seaweeds are considered as source of bioactive compound and produce great variety of secondary metabolite characterized by broad spectrum of biological activities. Species of brown seaweed are well known to contain large amount of alkaloid, saponin, phlorotannin, fucoxanthin, steroid and sulfate fucoidan, which have high potential as antimicrobial activities. This research aims were to (1) study the best solvent extraction method which produce high yield, phytochemicals content and antifungal activity against *Aspergillus flavus*, (2) study the best species brown seaweeds which show high yield, phytochemicals content, antifungal activity against *Aspergillus flavus*, and (3) study the IC₅₀ of antifungal activity from the best brown seaweeds species extract.

The results showed that, (1) Non polar extract was produced by nonpolar extraction (solvent-solvent extraction) showed the highest inhibition activity against *A. flavus*. In contrast, sulfate polysaccharide was produced by water boiling extraction method promoted the fungal growth, thus this method is not suitable to be used to attract bioactive compound from brown seaweeds, (2) The most potential species was *Turbinaria decurrens* and *Turbinaria conoides* with highest antifungal activity among species of brown seaweeds. This indicated that non polar extract from both of them also could be the most valuable for further utilization in plant protection field. Based on consideration from their phytochemical content and availability in nature thus *T. decurrens* was more favorable than *T. conoides*, (3) IC₅₀ of the best selective brown seaweed extract (*T. decurrens* extract) was 23.8 mg/mL. It showed 3.3 times weaker of antifungal activity toward Amistar (IC₅₀ 7.3 mg/mL) and 6.4 times weaker toward Manfer (IC₅₀ 3.7 mg/mL). The brown seaweed extract still could be utilized but less effective as antifungal agent to replace the synthetic utilization. More efforts are needed in the next research to boost its activity by combination with other strong natural fungicide or by searching another model of extraction method.

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P53

Degradation of *Fusarium* mycotoxins deoxynivalenol and zearalenone by cold atmospheric pressure plasma

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As it is well known that mycotoxins of all kinds are able to withstand a diversity of processes like e.g. distilling and are still present in the final products different approaches have been undertaken to decay these toxins [1]. As an opposite method to these processes mostly taking place in solutions the hereby introduced physically approach of detoxification of mycotoxins is taking much lesser machine effort.

As the plasma technology has been shown to be capable of the degradation of spores and hyphae on different surfaces by some colleagues [2] as well as by ourselves [3], we now like to present the atmospheric pressure plasma as a powerful tool for the disintegration of *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN).

In this investigation we especially examined the cold atmospheric dielectric barrier discharge in a direct (direct DBD) and a remote modus (DBD plasma jet). In spite of good results of other studies using argon or helium/oxygen admixtures [4] as a working gas for surface decontamination all of our experiments have been carried out using compressed synthetic air. We show that we are capable of reducing the amount of both mycotoxins DON and ZEN on the surface of our samples from several 100 µg/L to just a few µg/L depending on treatment time and power density. The decay rates have been evaluated by means of HPLC-MS/MS.

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P54

Detoxification of deoxynivalenol in transgenic *Arabidopsis thaliana*

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Deoxynivalenol (DON) is type B trichothecene that is the most common mycotoxin in small grains. DON-producing *Fusarium* species possess DON acetylation activity as a self protection mechanism (Kimura et al., 1998, McCormick et al., 1999). We expressed Tri101 gene derived from *F. graminearum* (FgTri101) that encodes enzyme converting DON into 3-O-acetyldeoxynivalenol (3ADON) in *A. thaliana*. The primary FgTri101-expressing plants (T0) were generated by *Agrobacterium*-mediated transformation using floral dip technique. The zygosity of T1 plants was determined by qPCR and confirmed by a segregation assay. The enzymatic assay was conducted for ten T2 plants at 25°C. Six plants showed a high efficacy in conversion of DON to 3ADON while wild type plants (WT) did not show any conversion. Enzymatic test on extracts of four T2 plants at 25°C and 37°C showed that the enzyme was active at both temperatures. Reduction of DON concentration by a factor of six was achieved; the concentration of 3ADON in transgenic plants was up to 11 mg/kg. In a phytotoxicity assay, wild type and T2 transgenic plants were cultivated on media with or without 25 µM DON supplement. After 12-day cultivation, the root length was measured. Transgenic plants were significantly more tolerant to DON than wild type plants although DON reduced the root growth of both transgenic and wild type plants. The study confirms a high effectiveness of FgTri101 in DON detoxification *in planta*, indicating potential of this approach for transgenic crops.

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P55

The Austrian methodology for the classification of the maize assortment regarding the susceptibility to ear fusariosis

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The *Fusarium* mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM) are known as the most dominant mycotoxins in Austria. Despite, the visual susceptibility for ear rot is part of the registration procedure of maize varieties the contents of mycotoxins were not considered up to now. In the food and feed supply chains maximum levels are set for food whereas guidance values were recommended for feed.

Due to often weak correlations between the mycotoxin contents and the visual affected cob surface within the present methodology the susceptibility to ear fusariosis is assessed by both the affected cob surface and the *Fusarium* mycotoxins contents. In total, about 1000 maize samples (from existing varieties and candidates) were taken annually at over 33 different locations within the three most important climatic zones in Austria. The samples were analyzed for DON, ZEA and FUM content by ELISA. In addition, maize cobs of all varieties were visually inspected and the average of affected cob surface was calculated. The data gathered is used for an improved variety assessment for the risk of *Fusarium* mycotoxins as well as a monitoring the mycotoxin contamination of freshly harvested batches.

The obtained results were set into relation to the calculated local median values of the mycotoxins contents and affected cob surface. The so calculated relative values are transferred into a 9-stage scale. The grades obtained at different locations were combined over different environments to a variety specific overall grade for each criterion. The final grade for each maize variety was calculated from these overall grades of each criterion. The present methodology was applied on the classification of maize varieties and their candidates in 2011-2013 and obtained comparable results to the former classification with a slightly wider genotypic differentiation on the 9-stage scale.

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Fate of the mycotoxins deoxynivalenol and enniatins when cooking pasta

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The fate of mycotoxins during processing can help risk assessors on the interpretation of data on occurrence of mycotoxins in raw materials. The behaviour of each mycotoxin in a process can vary due to molecular characteristics and nature of the treatment. Aim of this study was to assess the influence of pasta cooking on the occurrence of the mycotoxins deoxynivalenol (DON), enniatin A (ENNA), enniatin A1 (ENNA1), enniatin B (ENNB) and enniatin B₁ (ENNB₁). Three contaminated dry pasta samples containing both DON and ENNB and a blank dry pasta were selected. The samples were cooked (100 g to 500 ml tap water) for 10 min in a laboratory setting according to a pre-set protocol. Cooked pasta, the cooking water and the cooked pasta after rinsing were sampled. Samples were stored at -20°C and ground under liquid nitrogen before analysis. Samples were analysed in duplicate for DON, ENNA, ENNA1, ENNB and ENNB₁ using an in-house validated method (extraction with acidified acetonitrile/water (80%/20%) followed by filtration and separation/identification using LC-MS/MS with acidified eluents). The mass balance showed that all the mycotoxins were recovered either in the pasta or in the cooking/rinse water. Roughly 40% of the DON was drained to the water which is in agreement with literature^{1,3} since DON has a hydrophilic character. Hardly any enniatins were lost from the pasta, which is in accordance with their hydrophobic nature. It was not in accordance with the results of the study of Serrano *et al.* (2013) who found loss of enniatins when cooking a pasta resembling system spiked with enniatins². It cannot be excluded that changes in cooking conditions (times and rinsing intensity) may slightly modify the mycotoxin retention percentages.

¹ Brera C. et al. (2013) Food Control 32:309-312

² Serrano AB. et al. (2013) Food Chemistry 141:4215–4225

³ Visconti A. et al. (2004) Toxicology Letters 153:181-189

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Characterization of 27 mycotoxin detoxifiers and the relation with *in vitro* zearalenone binding

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Addition of mycotoxin detoxifiers to feed is a common practice to counteract the deleterious effects of mycotoxins on animal health. Although a variety of this kind of feed additives is available on the market, little is known about their detailed composition and physico-chemical properties. This lack of knowledge might pose some difficulties for researchers and users to compare and evaluate the efficacy and safety of these additives.

The present study describes the characterization of 27 commercially available mycotoxin detoxifiers collected from various sources in Flanders and The Netherlands. Characterization comprised XRD-profiling of the mineral content, determination of the cation exchange capacity and exchangeable cations, acidity, mineral fraction, humidity and swelling volume. The XRD- and mineral fraction data were analysed with principal component analysis and explorative cluster analysis which enabled the identification of three distinct groups: smectite based (n=19), non-smectite based (n=5) and organic based detoxifiers (n=4). In a second experiment, an *in vitro* zearalenone binding test was conducted using phosphate buffered saline at pH 2.5, 6.5 and 8.0. The zearalenone binding of the smectite-based group was related to the physico-chemical properties using a multivariate linear regression model. The binding in the non-smectite based and organic based groups was analysed using a one-way ANOVA.

A large variability in binding properties was present, especially in the smectite containing group. Additives with smectite-containing mixed-layered minerals and additives containing humic acids expressed the highest binding which amounted to over 90%. The retained multivariate linear model included exchangeable potassium ($p \leq 0.05$), moist content ($p \leq 0.05$) and mineral fraction ($0.05 \leq p \leq 0.1$), indicating a statistical relation of these parameters with the *in vitro* zearalenone binding.

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Evaluation of the efficacy of smectite clays, yeast cell walls and activated beta glucans on the toxicological effects of aflatoxins in weaned piglets

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The aflatoxin toxicity experiment was carried out with weaned piglets to evaluate the efficacy of an anti-mycotoxin additive (AMA) consisting of smectite clays, yeast cell walls and activated beta glucans. The study was conducted by Instituto SAMITEC (Brazil). The aflatoxins (B1, B2, G1 and G2) used on the experiment were produced by fungi of *Aspergillus parasiticus*, with 93.8% of the toxins as B1. A total of 30 male piglets with an average body weight of 12.56kg were used in five dietary treatments with six replicates each (1/pen). The treatments included (1) control, (2) control + AMA (0.5%), (3) control + 1 ppm of aflatoxin, (4) Treatment 3 + AMA (0.25%), and (5) Treatment 3 + AMA (0.5%).

The pigs received feed and water ad libitum. At day 28, the pigs were slaughtered to determine the relative liver weight and total plasma proteins. Compared to the control animals, aflatoxin addition significantly ($p < 0.05$) and severely reduced 28-day body weight (-14.53%), feed intake (-11.22%) and average daily gain (-26.23%), while increasing feed conversion ratio (+20.63%) and the relative liver weight (+30.80%). However, addition of the AMA significantly ($p < 0.05$) improved all the parameters above. There was no significant difference between the two inclusion levels though numerically the higher inclusion showed more pronounced effects. Compared with pigs with aflatoxins alone, AMA at 0.5% increased 28-day body weight (+14.19%), feed intake (+10.35%), and average daily gain (+28.89%), while significantly decreasing feed conversion ratio (-13.99%) and relative liver weight (-17.44%). AMA was able to offset all negative impacts due to aflatoxins and produce similar growth performance to the control without aflatoxins. It was concluded that the AMA consisting of smectite clays, yeast cell walls and activated beta glucans has demonstrated its efficacy in effectively offsetting the deleterious effects of the aflatoxins on weaned piglets.

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***Fusarium proliferatum* and fumonisins accumulation risk on garlic food chain**

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Fusarium proliferatum is an emerging, spreading worldwide pathogen of garlic, causing bulb rot. During the whole crop cycle, fungal presence is not associated to characteristic symptoms. During storage, infected bulbs, conversely, undergo to a slow deterioration process: at first brown depressed water soaked spots and, in extremely severe infections, the entire bulbs rot, reducing the garlic shelf-life. The disease leads to appreciable loss of product. This fungus is well known to produce fumonisins. White garlies, cultivated in different northern Italian regions are subjected to mycological and HPLC analysis, to detect the pathogen and quantify the mycotoxins. Basal plate of garlies were dissected and cultured in PDA Petri dishes amended with streptomycin and neomicyn sulphate. Strains morphologically belonging to *F. proliferatum* species were tested by PCR with specific primers and with primers for Fum1 gene involved in fumonisin production. For mycotoxin analysis, cloves were dried for one week at 40°C, grinded in fine powder and analyzed by IAC/HPLC. *F. proliferatum* is present in all the tested bulbs and all the strains collected show the presence of FUM1 gene. The fumonisin levels in cloves ranged from 0.05 ppm to 0.68 ppm for B1 and up to 0.1 ppm for B2. These quite low levels are reached within a short period of conservation in comparison to the time from storage to consumption.

Mycotoxin levels should be checked in different food chain steps and on garlic derived products, therefore it is necessary to consider the influence of the conservation time at home, by consumers to better evaluate the possible risk in human health.



Figure. Symptoms of *Fusarium proliferatum* rot on cloves

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Identification of *Fusarium* spp. and mycotoxins on *Allium* spp. in Germany

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The most important *Fusarium* species causing *Fusarium* basal rot of onion is *Fusarium oxysporum* in Germany. However, in Mediterranean countries like Israel the *Fusarium* salmon blotch caused by *F. proliferatum* is more important than *F. oxysporum*. The aim of the survey was to evaluate different *Fusarium* spp. infecting onions and show the increasing importance of mycotoxin producing fungi like *F. proliferatum* in Germany. In cooperation with a breeder, consultants and producers seeds and sets of different *Allium* spp. as well as *Allium cepa* bulbs from different fields in Northern and Southern Germany were sampled and analyzed in 2013. Different *Fusarium* spp. were isolated from onions and identified by morphological characterization.

Up until now 19 *Fusarium* spp. were identified. The most common species are *F. oxysporum*, *F. solani* and *F. proliferatum*. *F. oxysporum* was detected in all sampled onion field and it is the main *Fusarium* sp. in Germany. *F. solani* is the second most common *Fusarium* species isolated from *Allium* sp. In about half of the sampled onion fields *F. proliferatum* was detected.

Interestingly, high mycotoxin producer such as *F. proliferatum* and *F. sporotrichioides* occurred in German onion fields. Also, *F. poae* was isolated for the first time from German onions. The diversity of *Fusarium* spp. is higher on young sets compared to the older bulbs. At least two different *Fusarium* spp. were present at each sampled onion field. The three main *Fusarium* spp. are able to produce mycotoxins like beauvericin, enniatin, fumonisin, fusaproliferin, moniliformin and T-2 toxin. The variety of mycotoxins in onions will be quantified by ongoing mycotoxin analysis.

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Assessment of mycotoxin occurrence in feed samples from the veal calf industry

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The veal industry specializes in rearing dairy and beef calves on a controlled low-iron diet to obtain white veal meat. The calves are fed a milk replacer, mainly based either on skimmed milk powder or on vegetable protein. In recent years, roughage and low-iron concentrates have been added to the diet to ensure better rumen development and a better welfare for the animals. This study evaluate the mycotoxin contamination of feed samples from the veal industry. In this pilot study, 45 feed samples were collected from 15 different veal farms in Belgium. On each farm 3 to 6 samples per feed component, respectively milk replacer, roughage (straw or corn silage) and concentrate feed, were collected and pooled per feed component. Samples were analysed by validated multi-mycotoxin (UHP)LC-MS/MS methods. About 13% of the milk replacer samples were contaminated with fumonisins FB1 and FB2, with an average contamination level of respectively 32 ± 7 and 13 ± 1 µg/kg. None of the other mycotoxins included in the detection method were found. However, all roughage and concentrate feed samples were contaminated with at least one mycotoxin. Deoxynivalenol (DON) was most prevalent, contaminating 80% of the roughage samples (637 ± 621 µg/kg, max. 1818 µg/kg), and all concentrate samples (411 ± 156 µg/kg, max. 693 µg/kg). Also the DON conjugates 3- and 15-AcDON were present in 40% of the feed samples. Besides DON, also enniatin B was highly prevalent (73% of the samples). Other mycotoxins detected were alternariol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, neosolaniol, nivalenol and zearalenone. This survey demonstrates a multi-mycotoxin contamination in veal feed samples, especially in roughage and concentrate feed. The contamination levels are comparable with previous published mycotoxin surveys in feed for other animal species. Mycotoxin occurrence in veal feed will not affect food safety. Further research is necessary to evaluate the effect of mycotoxins on animal performance.

G. Antonissen and B. Valgaeren contributed equally to this work. The authors acknowledge the technical assistance of J. Muyle and S. Degroote, the support from cooperating veterinarians and integrations.

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Immunochemical assessment of alternariol in fruits, vegetables, and products thereof

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The mycotoxin alternariol (AOH) is produced by different species within the genus *Alternaria*. AOH was found to be a natural contaminant in cereals, olives and nuts. Recently, the European Food Safety Authority (EFSA) stated the need for more data on AOH occurrence in food. Because the average daily per capita consumption of fruits, vegetables and products thereof is very high, even low contamination levels may be of relevance if the contamination frequency is high. The aim of the present study was the examination of fruit and vegetable juices (fruit juices, carrot juice), and of infant food from the German market. Two highly sensitive enzyme immunoassays, based on either monoclonal (mAb) or polyclonal (pAb) antibodies against AOH, were used to develop simple analytical methods for AOH in these matrices, which involved either liquid-liquid partitioning with ethyl acetate (carrot juice, infant food) or dilution with phosphate buffered saline (juices).

Detection limits for the different matrices were in a range of 0.6-1 ng/g, mean recoveries (1-50 ng/g) were 77.7-121 % (pAb) and 61.2-90.0 % (mAb), respectively.

AOH was found frequently in cherry juices, orange juices, and multi-fruit juices. Especially cherry juices were all positive for AOH, which is in agreement with literature data. AOH was detected in a concentration range between 0.74 ng/g and 27 ng/g. Obviously the production of cherry juice has an inherent problem with - either preharvest or postharvest - contamination by AOH-producing fungal species. In infant foods, some samples showed positive results in the mAb EIA, but compared to the results of the pAb EIA, no detectable amounts of AOH could be found. This indicates that an AOH-like, cross-reacting compound may have been responsible for the positive results in the mAb EIA. Further studies should aim in a validation of the developed methods and in further studies on the occurrence of AOH in food and feed.

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Immunochemical studies on the occurrence of isofumigaclavine A in mouldy cheese during storage

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Storage of cheese under suboptimal kitchen hygiene conditions might be associated with unwanted fungal growth. One of the most common “household moulds” is *Penicillium roqueforti*. Although it is common knowledge that unwanted secondary mould on cheese may be harmful, no hard data on mycotoxin production under real life conditions could be found. We therefore tested the ability of wildtype household fungi to produce mycotoxins under “real life” (and arbitrary) household conditions, without specifically inoculating cheeses with pure fungal culture. Isofumigaclavine A (IsoFuA, synonym: Roquefortin A) was used as marker toxin. IsoFuA is a clavine type ergoline derivate and therefore shows structural similarities to ergot alkaloids. An enzyme immunoassay for ergot alkaloids detecting the generic ergoline ring system was used for determination of IsoFuA, using a standard curve established for this toxin.

A total of 46 cheese samples (semihard and hard cheeses, pasta filata, all produced without fungal mycelium) were purchased from regional supermarkets. Prepacked cheeses were shortly opened in a typical kitchen environment and then immediately closed again. All samples were stored at 7 °C until visible fungal growth became apparent. Additionally, five samples of semihard and hard cheeses with - unwanted - fungal growth were provided by different households for analysis. Fungal material from mouldy cheeses was cultured on malt agar and roughly classified into four groups, depending on their macroscopical appearance. In cheese samples with typical signs of grey or grey-blue mycelium, infected areas contained detectable levels of IsoFuA . Maximum levels of IsoFuA in visibly mouldy cheeses (surface area) were 3 µg/g. IsoFuA-positive results were only obtained for areas of visible fungal growth, cheese material distal from mouldy parts was all IsoFuA-negative. In conclusion, fungal infestation of cheeses under real life household conditions may indeed be associated with IsoFuA production at high levels. Because this is indicative for *P. roqueforti*, other mycotoxins produced by this fungus, such as mycophenolic acid, presumably will appear as co-contaminants.

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Screening of 72 maize samples from Austrian harvest 2013 for more than 380 mycotoxins and secondary metabolites

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Due to the natural co-occurrence of mycotoxins, the toxicity of contaminated feed cannot be accurately estimated by determining the concentration of one or two toxins only. In an effort to further broaden the knowledge on mycotoxin occurrence and co-occurrence in feed, a LC-MS/MS method (Vishwanath et al. 2009) was used to screen 72 maize samples for more than 380 mycotoxins and other secondary metabolites. The 72 maize samples were collected from Austrian harvest in 2013.

In 72 samples between 16 and 84 metabolites could be detected per single sample - all together 128 different substances. The mycotoxins, which were found most often were beauvericin, enniatin B/B1, aurofusarin, and culmorin (100% of the samples) followed by zearalenone (mean of positives 111 µg/kg; max. 1880 µg/kg), enniatin A1, moniliformin, and equisetin (99% of the samples). 96% of the samples were found positive for deoxynivalenol (mean of positives 593 µg/kg; max. 3105 µg/kg) and nivalenol (mean of positives 27 µg/kg; max. 108 µg/kg). Deoxynivalenol-3-glucoside always co-occurred with deoxynivalenol.

Above that, 54% of the samples were positive for HT-2 toxin (mean of positives 25 µg/kg; max. 216 µg/kg) and 31% for T-2 toxin (mean of positives 10 µg/kg; max. 44 µg/kg).

The results of the analysis of 72 Austrian maize samples with a multi-mycotoxin method based on LC-MS/MS clearly showed that mycotoxin co-contamination is the rule. In order to permit an accurate assessment of the risks associated with the presence of these contaminants more data is needed on their metabolic fate, toxicities and possible synergistic interactions with other mycotoxins present.

Reference:

Vishwanath V, Sulyok M, Labuda R, Bicker W, Krska R (2009) Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. *Anal Bioanal Chem* 395:1355-72.

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Occurrence of fungi and their metabolites in porridges intended for infants and young children

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Cereal products for infants and young children must meet a number of strict requirements regarding the composition, nutritional value and health quality. Baby food should be produced from the highest quality raw materials, as cereals may be contaminated with fungi and their toxic metabolites – mycotoxins.

Material for study consisted of 21 porridges of 3 different manufacturers, purchased in retail trade in Bydgoszcz. The samples were divided into 2 groups: cereal porridges (n=7) and milky-cereal porridges (n=14), and subjected to mycotoxicological and mycological analyses. Fumonisin were evaluated in samples contained corn (n=9). Trichothecenes, zearalenone and fumonisin were determined using SPE columns for sample preparation and HPLC-MS/MS. Aflatoxins (AF) and ochratoxin A (OTA) were isolated with IAC and determined with HPLC-FLD. Moulds and yeasts were determined using both YGC and DG18 medium.

Mycological analysis revealed that the predominant group of all fungi were pathogenic molds *Penicillium* (max 914 cfu/g, DG18 medium). Additionally, the molds of the genera: *Aspergillus*, *Mucor*, *Rhizopus* and *Cladosporium* were identified. Of all the analyzed samples only 3 were not contaminated with molds. Yeast were present in one porridge sample at a low level (3 cfu/g). Mycotoxins evaluation results have been presented in Table 1. In none of the analyzed samples mycotoxins level exceeded the acceptable value set by the Commission Regulation (EC) No 1881/2006.

Table 1. Mycotoxins contamination of infant and young children porridge samples

Mycotoxins content in baby porridge samples (ppb)		DON	NIV	HT-2	T-2	ZEN	FUM	OTA	AF
Cereal porridges	%Contamination	71	14	57	57	71	0	0	0
	Mean	12.5	nd	<2	<0.6	<0.2	nd	nd	nd
	Median	8.15	nd	<2	<0.6	<0.2	nd	nd	nd
	Min	nd	nd	nd	nd	nd	nd	nd	nd
	Max	31.1	<3	<2	<0.6	0.32	nd	nd	nd
Milky-cereal porridges	%Contamination	93	14	29	14	100	0	7	0
	Mean	13.6	nd	<2	nd	0.32	nd	nd	nd
	Median	6.02	nd	nd	nd	0.32	nd	nd	nd
	Min	nd	nd	nd	nd	<0.2	nd	nd	nd
	Max	86.0	<3	7.42	0.96	0.59	nd	<0.4	nd

nd – not detected

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The occurrence of moulds of the *Trichoderma* genus and other moulds in the compost for *Agaricus bisporus* growth

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The conditions in the production halls of *Agaricus bisporus* i.e. high temperature and high humidity of the compost and the air are preferable not only for the growth of champignons, but also for the development of pathogenic fungi. In mushroom growing facility located in Kujawy-Pomerania region mycological survey of compost was conducted in order to explain in the causes production of loss. The cultivation of mushrooms was conducted in phase II compost (so-called "dice"). In case of phase II compost the building itself or the compost, can be the source of infection. Therefore, it is necessary to implement strict sanitation and disinfection procedures. The development of *Trichoderma* is usually associated with compost overgrowth delay and the destabilizing of its selectivity. Moulds that grow intensively take up space in the compost degrading its quality by releasing toxic compounds, thus infecting mycelium and fruiting bodies of the mushrooms. Highly contaminated compost becomes barren and considerable increase in the temperature of the compost is observed. The study material was the compost purchased from three companies: F1 (n = 14), F2 (n = 7), F3 (n = 1). The samples for the study were collected after 2 weeks following the lining of the compost in the production halls, but before applying the peat cover. Mycological examination was carried out on YGC agar medium. The results are expressed as the number of colony forming units per gram of a sample. The table 1 shows the obtained results.

The highest numbers of moulds was detected in the compost purchased from F1, with pathogenic *Trichoderma* making up 80% of the total number of molds. In the F2 group moulds of the genus *Trichoderma* represented 43% of all moulds. In the F3 group *Aspergillus* constituted 100% of the moulds detected.

Table 1. Total number of fungi, moulds and yeasts in the analyzed samples of the compost used for button mushrooms growth.

Material	General number of fungi [CFU/1g]	General number of moulds [CFU/1g]	General number of yeast [CFU/1g]
F1 (n=14)	$1,3 \times 10^5$	$1,2 \times 10^5$	$2,4 \times 10^4$
F2 (n=7)	$1,0 \times 10^5$	$1,0 \times 10^5$	$6,2 \times 10^2$
F3 (n=1)	$1,3 \times 10^5$	$1,3 \times 10^5$	$9,1 \times 10^2$

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Deoxynivalenol and zearalenone occurrence in Polish beers

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Beer is the oldest alcoholic beverages consumed on a regular basis by a large number of people in the world. Brewing and fermentation of starches mainly derived from cereal grains, particularly barley, wheat and maize. One of the challenges is that cereals used during the production of beer are frequently contaminated with mycotoxin-producing fungi, either in the field, storage or during the malting stage.

The main research in this area has been focused on deoxynivalenol and zearalenone. These mycotoxins belong to a major class of *Fusarium* toxins. Deoxynivalenol also known as vomitoxin, mainly associated with feed refusal and emetic in animals and inhibits the synthesis of DNA, RNA and protein. Zearalenone is of low toxic effect but, together with its metabolites, it can strongly bind estrogen receptors, resulting in reproduction problems in animals.

The study describes the occurrence of deoxynivalenol and zearalenone in a samples of 37 different bottled commercial Polish beers collected from retail outlets. The analysis was conducted using an enzyme-linked immunosorbent assay (ELISA) method, Ridascreen Deoksynivalenol and Ridascreen Zearalenone (R-Biopharm).

DON was found in 100% (25,94 – 506,90 µg/L) and ZEA in 84% (0 – 1,71 µg/L) of tested beers. DON and ZEA were found in Polish beers at mean level respectively 171,97 µg/L and 0,63 µg/L, which contribute an average of 71,58% and 0,57% of the estimated tolerable daily intake of both mycotoxins considering a daily intake of 0,25 l of beer per capita in Poland. Concentrations of DON have been found to be slightly higher in unpasteurised beers comparing to pasteurised, however for ZEA concentration the situation was inverse. Similar results were obtained comparing mycotoxins concentrations in top-fermented and bottom-fermented beers. The ZEA content was also higher in pasteurized dark-coloured beers. The co-occurrence of *Fusarium* mycotoxins were mainly observed in beers with higher alcohol content (≥6,8%).

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Occurrence of (masked) *Alternaria* toxins - A survey in foodstuffs commercially available on the Belgian market

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The fungal genus *Alternaria* contains numerous species that can contaminate a wide variety of crops in the field and cause post-harvest decay of various fruits, grains and vegetables. In addition to causing economic losses, *Alternaria spp.* can form mycotoxins under certain conditions. Due to the possible risk for public health related to the presence of *Alternaria* toxins in food, EFSA stipulated that additional quantitative occurrence data are urgently needed to refine exposure assessment. Furthermore, *Alternaria* toxins can in line with other xenobiotics be partly metabolised, which may lead to the formation of conjugated metabolites in plants. These "masked" mycotoxins are of human health concern as they may be capable to release their native precursors in the digestive tract of organisms. Therefore, a fast and sensitive UPLC-ESI[±]-MS/MS method for the determination of free (AOH, AME, ALT, TeA, TEN & ATX I) and conjugated (AOH- and AME-3-sulphate, AOH- and AME-3-glucoside) *Alternaria* toxins in multiple matrices, such as cereal products (rice, oat flakes), beer, fruit and vegetable juices (carrot, apple and grape juice), tomato products, lentils, sesame- and sunflower oils/seeds was developed and validated (in agreement with the criteria mentioned in Regulation 401/2006/EC; Commission Decision 2002/657/EC). The method, applying isotopically labelled internal standards, allowed for the simultaneous determination of 10 *Alternaria* toxins in a one-step chromatographic run (7 min). Minimal sample clean-up was carried out on the different matrices, making it possible to perform sampling, analysis and finally detection and quantification in less than 24h. Yet, low limits of quantification (<1-5 µg.kg⁻¹) were obtained for all matrices. Subsequently, 200 samples of a variety of commercially available foodstuffs were analyzed between February 2013 and April 2014.

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P69

***Fusarium* toxins in wheat and ergot alkaloids in rye of the federal state Brandenburg harvested 2013**

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To record the quality of wheat and rye samples, collected from farmers of all the agricultural districts of the federal state Brandenburg with special consideration of organic cultivation, were analysed for *Fusarium* toxins (2000-2013) and for ergot alkaloids (2012-2013). DON was detected in wheat for all years of the investigation except in 2010 (5-86% positive samples, maximum levels: 50-10,400 µg/kg). ZEA, NIV, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside were rarely detected, T-2-/HT-2 toxins, DAS and FUS-X only occasionally or not at all. 2013 DON was contained in 26% of the wheat samples (maximum 2477 µg/kg; mean/median of the DON-positive 556/290 µg/kg). ZEA was detected only in 3 % of the samples (max. 33µg/kg). The 2 wheat samples which exceeded the EU maximum limit of 1,250 µg/kg originated from integrated cultivation. 30% of the wheat samples from integrated cultivation contained DON (mean/median: 629/409 µg/kg), but only 2 of the 15 organic wheat samples (maximum 101 µg/kg, mean/median 78 µg/kg). Ergot alkaloids were detected in rye samples in 2012 very rarely. 2013 was a year with frequent occurrence (67% positive samples) and high ergot alkaloid contamination (up to 4,850 µg/kg; mean/median of all positive samples: 536/237µg/kg). The individual ergot alkaloids occurred relatively evenly distributed. A «leading alkaloid» could not be seen. In the positive samples 2-12 ergot alkaloids were detectable. The highly contaminated samples contained 10-12 ergot alkaloids, samples with medium content 4-12 ergot alkaloids. The correlation between the content of ergot sclerotia and the content of ergot alkaloids was low ($r=0,51$). Differences between integrated and organic cultured rye samples were observed. The highest contents of ergot alkaloids were measured only in samples from integrated cultivation (74% positive sample; mean/median: 620/260 µg/kg). The maximum content of the samples from organic cultivation was 776 µg/kg (48% positive sample; mean/median: 281/197 µg/kg).

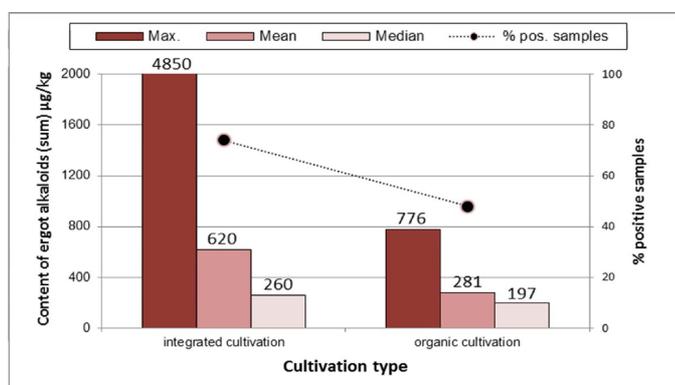


Figure. Maximum, mean and median of rye samples in response to cultivation type.

The project was supported by the Ministry of Infrastructure and Agriculture of the federal state Brandenburg.

P70

***Alternaria* mycotoxins in freshly harvested winter wheat kernels 2001-2010**

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Although, the genus *Alternaria* is considered to be an important plant pathogen and capable to produce mycotoxins, the presence of *Alternaria* mycotoxins in cereals has been largely ignored in Europe. This is very likely due to a lack of survey data. This study aimed at filling this gap by investigating a total of 1064 freshly harvested winter wheat samples from commercial farms in different regions of the State of Brandenburg (Germany) in the years from 2001 to 2010 (1). We analysed alternariol (AOH), its monomethylether (AME), altenuene (ALT, since 2006) and tenuazonic acid (TeA) by a HPLC method with diode array and fluorescence detection. The most frequently found *A.* mycotoxin was TeA. An amount of 322 out of 1064 samples (30.3%) were naturally contaminated by TeA, 86 out of 1064 by AOH (8.1%), 33 out of 1064 by AME (3.1%) and 7 out of 267 samples (2.6%) were contaminated by ALT. The maximum toxin contents in all years were 4,224 µg TeA kg⁻¹, 832 µg AOH kg⁻¹, 905 µg AME kg⁻¹ and 197 µg ALT kg⁻¹. In each year, TeA was detectable in wheat ears. The proportion of positive samples was conspicuously different: from only 1-4% in 2001 and 2008 up to 100% in 2009 and 2010. A co-occurrence of several *A.* mycotoxins in wheat samples was unfrequent: only three samples were contaminated by all the four toxins, 14 by three toxins, 61 by two toxins and 273 samples by only one toxin. The combined detection of TeA and AOH was the most frequent case, an exclusive co-occurrence of AOH and AME was not detected. The contamination of wheat ears in the state of Brandenburg in the wet years 2010, 2009 and 2002 were most pronounced, whereas 2001 and 2008 were *A.* “toxin free” years. The accumulation of TeA in freshly harvested wheat kernels seems to depend on preceding crop and tillage. Minimum tillage practices and maize as well as winter wheat as preceding crops led to increased TeA concentrations in wheat. Furthermore, differences in the susceptibility between the cultivars towards *A.* infection and a successional mycotoxin accumulation were not detected.

1 Müller MEH, Korn U (2013) *Alternaria* mycotoxins in wheat – A 10 years survey in the Northeast of Germany. Food Control 34: 191-197.

P71

Occurrence of aflatoxin M1 in raw milk produced in Qena (Egypt)

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Aflatoxins are natural toxic compounds produced by fungi such as *Aspergillus flavus* and *Aspergillus parasiticus*. Due to higher temperature and humidity, fungi are continuously grown and contaminated cereal grains with aflatoxin B1 (AFB1). Dairy cows fed AFB1 contaminated feeds can produce milk contaminated with AFM1. Therefore, the aim of this study was to investigate whether raw milk produced in Qena province (south of Egypt) is contaminated with AFM1.

A total of 48 raw milk samples were collected from dairy farms in Qena and analysis with ELISA for the presence of AFM1. The results showed that the occurrence of AFM1 was 97.9 % (47 samples out of 48 samples were positive) and the mean level of AFM1 was 62.8 ± 32.1 ng/l ranging from 2 ng/l (minimum value) to 110 ng/l (maximum value). Moreover, the level of AFM1 in 47 (53.2 %) of raw milk samples was higher (79.9 ± 17.3 ng/l) than the maximum tolerance limit (50 ng/l) established by European Union (EU) and the amount was ranged from 51-110 ng/l. The higher occurrence of AFM1 led to suggest the contamination of raw milk is very high probably due to the higher contamination of cattle feeds with AFB1 in south Egypt.

In conclusion, more attention is required for the contamination of raw milk produced in Qena (Egypt) with special focus on occurrence of AFB1 in the feed offered to dairy cows. Therefore, there is a special concern for regulations of mycotoxins levels not only in human foods but also in animal feeds.

P72

Ochratoxin A (OTA) transfer from ground coffee to coffee drink according coffee preparations

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OTA contaminates foodstuffs from both plant and animal origins. This mycotoxin is nephrotoxic, hepatotoxic, teratogenic, neurotoxic, immunotoxic, carcinogenic (Malir F, Ostry V, Novotna E (2013) Toxicity of the mycotoxin ochratoxin A in the light of recent data. *Toxin Rev* 32: 19–33). This study investigates the OTA content in ground and roasted coffee samples and the OTA passage into coffee drinks. Validated and accredited HPLC-FLD modified method with limit of quantification of 0.35 ng/g was employed for OTA determinations (Creppy EE, Castegnaro M, Grosse, et al. (1993) Etude de l'ochratoxicose humaine dans trois regions de France. *Human ochratoxicosis and its pathologies* 147–158). The samples of coffee were extracted and cleaned by means of SPE columns (Strata Phenyl, Phenomenex, USA) and OCHRAPREP® columns (R-Biopharm, Germany) (Tozlovanu M, Pfohl-Leszkowicz A (2010) Ochratoxin A in roasted coffee from French supermarkets and transfer in coffee beverages: comparison of analysis methods. *Toxins* 2: 1928-1942). One ground coffee naturally contaminated (NC) at 0.92 ng/g and one ground coffee artificially contaminated (AC) at 5 ng/g have been used to prepare coffee beverages according different kind of processes. The highest percentage of OTA in beverage was in false Turkish coffee: 66.1 % ±1.82 % in NC versus 64.9 % ±1.65 % in AC; followed by Turkish coffee 51.7 % ±2.10 % (NC) vs 52.4 ±0.7 % (AC); (using the automatic coffee machine, /EA 100, AEG/ it was prepared:) Lungo: 54.5 % ±2.42 % (NC) vs 53.4 ±2.50 % (AC); Americano: 50.8 % ±2.28 % (NC) vs 49.8 % ±3.26 % (AC); Espresso: 32.2% ±3.65 % (NC) vs 32.4% ±4.1 % (AC); Doppio: 30.2 % ±2.23 % (NC) vs 30.2 % ± 3.44 % (AC), Ristretto: 22.3% ±3.64 % (NC) vs 24.5% ±1.35 % (AC). However, for more accurate toxicological assessment determination of other ochratoxins, i.e. OTB (dechlorinated OTA), OTC (ethylated OTA) and some metabolites, such as OP-OTA or OTHQ should be done.

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P73

Biomonitoring of mycotoxins in urines of mill workers from North Rhine-Westphalia

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As grains are often contaminated with mycotoxins there is dietary exposure from food commodities in the general population. Processing of grains such as milling may result in an additional exposure to airborne mycotoxins (Degen GH, 2011, World Mycotoxin Journal 4:315-327). In a pilot study urinary biomarkers were analyzed to investigate possible workplace exposure in mill workers in relation to a control group with dietary mycotoxin intake alone.

Spot urines of 18 workers from 3 grain mills in North Rhine-Westphalia, Germany, were collected during their shift; urines from 13 volunteers (IfADo staff) with matched age structure served as controls. Deoxynivalenol (DON), zearalenone (ZEN), citrinin (CIT) and ochratoxin A (OTA) were measured with sensitive LC/MS-MS and HPLC-FD methods after sample clean-up by immunoaffinity columns (DON, CIT, ZEN) or liquid-liquid-extraction (OTA). Also metabolites were analyzed: Deepoxy-deoxy-nivalenol (DOM-1), dihydrocitrinone (OH-CIT), OT α , α - and β -zearalenol. To include phase-II metabolites, enzymatic hydrolysis with β -glucuronidase/arylsulfatase was performed prior to sample clean-up. Urine analyte concentrations were adjusted for creatinine (crea) content to allow comparisons between spot urine samples.

CIT, DON, OTA and ZEN were detected in nearly all urines from mill workers and controls. The metabolite OH-CIT was often found in higher concentrations than CIT (~0.14 and 0.045 $\mu\text{g/g}$ crea, respectively), pointing to an effective detoxication of CIT in humans (Föllmann et al., 2014, Arch Toxicol, DOI: 10.1007/s00204-014-1216-8). DON was detected in the highest concentrations (~6 $\mu\text{g/g}$ crea) followed by OTA (~0.1 $\mu\text{g/g}$ crea); ZEN and its metabolites appeared at lower concentrations (~0.04 $\mu\text{g/g}$ crea). DON and DOM-1 levels in the two cohorts were comparable. For CIT, OH-CIT, OTA and ZEN slightly higher concentrations were found in the mill worker urines. But this difference was not significant.

We conclude that the levels of mycotoxins measured in all urine samples reflect mainly dietary exposure of the two cohorts. An additional occupational (inhalational) exposure of mill workers if any is very low at the investigated workplaces.

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P74

Determination of total aflatoxins and aflatoxin B1 content in fruits and oleaginous seeds marketed in Transylvania

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A very important category of mycotoxins are aflatoxins, made by *Aspergillus* fungi (*A. flavus*, *A. parasiticus* and *A. nomius*) (Galvano, 2001). From all aflatoxins, the following stand out: aflatoxin B1 - most toxic one, aflatoxin B2, G1 and G2 (Masoero, 2009). The aflatoxins can enter the body direct via cereals, seeds, spices, fruits and other plant materials and indirectly through the food produced by animals (meat, milk, eggs and their derivatives) whose feed was contaminated (Galvano, 2001). A total number of 25 samples of fruit and seeds originating from supermarkets and small shops around Transylvania were investigated. Organoleptic and mycotoxicological tests were performed for all samples. For mycotoxicological testing two immunoenzymatic competitive test for quantitative determination of aflatoxins from aliments and cereals were used: RIDASCREEN®FAST for total aflatoxins and RIDASCREEN Aflatoxin B1, for aflatoxin B1. Subsequent organoleptic testing, 2 samples were noticed to have modified characteristics.

Mycotoxicological testing for total aflatoxins revealed that 24 % of the total investigated samples have shown values over the admitted limit in the EU Legislation. From the total positive samples, 6 were with higher than accepted total aflatoxin content, and 2 samples contained aflatoxin B1 above the upper limit established by the European legislation. The highest total aflatoxin and aflatoxin B1 levels were found in roasted corn (46.5 ppb for total aflatoxins, respectively 4.01ppb for aflatoxin B1).

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P75

Occurrence of macrocyclic trichothecenes in German straw

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Stachybotrys spp. are filamentous fungi with cellulolytic properties. *Stachybotrys chartarum* chemotyp S is able to produce highly cytotoxic macrocyclic trichothecenes (roridin E, roridin L-2, satratoxins F, G and H and verrucarins J) (Hinkley SF, Jarvis BB (2001): Chromatographic Method for Stachybotryotoxins. Mycotoxin Protocols. Springer:173-194). The natural occurrence of macrocyclic trichothecenes has been described in straw which is frequently used as animal bedding material or for animal feed. Macrocyclic trichothecenes are responsible for causing a serious disease called stachybotryotoxicosis which was described after contact with or ingestion of contaminated straw (Harrach B, Bata A, Bajmocy E, Benko M (1983): Isolation of satratoxins from the bedding straw of a sheep flock with fatal stachybotryotoxicosis. Applied and environmental microbiology 45 (5):1419-1422; Danko G (1975): Stachybotryotoxicosis and immunosuppression. International Journal of Environmental Studies 8 (1-4):209-211).

In order to investigate the current status of straw quality, 29 samples were analyzed for various macrocyclic trichothecenes by LC-MS/MS. All straw samples were of the crop years 2007 and 2008 and were collected according to the German official directive for feed sampling (VDLUFA) in different German federal states.

In one straw sample macrocyclic trichothecenes were detected by ELISA screening (Enviroligix Quantitox™). Confirmation by LC-MS/MS showed the presence of roridin E, roridin L-2, satratoxins F, G and H and verrucarins J. The sum amount of macrocyclic trichothecenes was 290 µg/kg quantified by LC-MS/MS and 225 µg/kg roridin A-equivalents in the ELISA-test. This result shows that macrocyclic trichothecenes can be found in field samples highlighting that stachybotryotoxicosis still represents a risk for animal health.

P76

Worldwide occurrence of mycotoxins in feeds and feed components in the year 2013

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In 2013 a follow-up (start in 2004) on a worldwide survey about the most important mycotoxins in feedstuffs was conducted to collect information on the presence of mycotoxins in commodities most commonly used for feed production in farm animals. A total of 4,218 samples (mainly corn/corn silage, soybean/soybean meal, wheat, finished feed and other grains) sourced in America, Europe and Asia were analysed for the presence of the following mycotoxins including aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA).

Samples were analysed by high performance liquid chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA). Only single commodities were analysed by ELISA. More complex matrixes which could interfere with the ELISA method such as DDGS and finished feed were analysed by HPLC. For the purpose of data analysis, non-detect levels are based on the quantification limits (LOQ) of the test method for each toxin.

In the more than 4,000 samples analysed worldwide, Afla were present in 30%, ZEN in 37%, DON in 59%, FUM in 55% and OTA in 23%. Average contamination levels of all samples were 10 ppb for Afla, 49 ppb for ZEN, 458 ppb for DON, 778 ppb for FUM and 2 ppb for OTA. 19% were tested negative for the presence of five investigated mycotoxins. Thirty six percentages showed presence of one of them, and two or more of the tested mycotoxins were present in 45% of the samples.

Results of this survey highlighted the necessity of mycotoxin testing prior to the feeding of animals. More than 80% were positive for at least one mycotoxin. The presence of more than one mycotoxin in half of the samples draws attention to the multi-mycotoxin contamination. The results underline the necessity of constant mycotoxins monitoring in feedstuffs and a proper mycotoxin risk management.

P77

Do endophytic entomopathogenic fungi produce mycotoxins when growing inside plant tissues?

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It has been shown only recently that entomopathogenic fungi, such as *Beauveria bassiana* or *Metarhizium anisopliae*, are also acting as biological control agents against herbivorous insects when endophytically colonizing plant tissues. However, because these entomopathogenic fungi are known to produce several mycotoxins, including beauvericin, destruxin A, D and E, caveats have been issued that these toxins would accumulate in the tissues during growth of inoculated plants. This would then interfere with human consumption and would constrain their use in a biological control strategy. It is therefore of prior importance to understand the interaction between plants and endophytic entomopathogenic fungi metabolism. We used endophytic isolates of *Metarhizium anisopliae* (150 and 153) or an endophytic isolate of *Beauveria bassiana* to inoculate tomato or cotton plants either via seeds or roots. Tomato plants were also co-inoculated with *Phytophthora infestans* to understand the role of multiple infections on mycotoxin metabolism. Following the inoculation newly emerged unfolded leaves were sampled at the seventh leaf growth stage and extracted by methanol/water for the analyses of mycotoxins, using the reversed phase liquid chromatography LC-MS/MS.

Our results demonstrate that the endophytic growth of entomopathogenic fungal isolates used in this study inside the tissues of plants does not correlate to any mycotoxin production emphasizing that these endophytic fungal strains can be considered as a promising biological control strategy for several major pests and diseases in some cash crops.

P78

Variability in accumulation of mycotoxins and susceptibility of Polish winter wheat breeding lines to *Fusarium* head blight

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Fusarium head blight (FHB) caused by *Fusarium* species is one of the most important cereal diseases in Poland. The aim of our study was to evaluate selected winter wheat breeding lines and varieties in terms of mycotoxin accumulation and resistance to FHB. In 2013, 51 breeding lines and varieties representing different genetic background were tested in environments of Central Poland (Radzikow) and Western Poland (Cerekwica). Field trials were conducted in 3 replicates. Wheat heads were inoculated twice at flowering stage with suspension of 3 isolates of *F. culmorum* with 1-week interval. *F. culmorum* isolates produced DON, NIV and ZEA. Disease incidence and severity were rated twice, after 2 and 3 weeks after inoculation and FHB indexes (FHBi) were calculated.

In harvested grain share of damaged kernels (FDK%) was assessed and mycotoxin content (DON, NIV ZEA) was analyzed with ELISA and GC-ECD methods.

FHBi in both locations were similar (3,3-48,7% (mean 19,9%) and 4,8-56% (mean 22,2%) in Radzikow and Cerekwica respectively). Similar variability of this trait was observed (47 and 49,3% respectively). FDK was significantly higher in Western Poland than in Central Poland (57,4 and 28,4% respectively). Mycotoxin content was also much higher in Western Poland. Respective average values for DON, NIV and ZEA content (20,3 ppm, 18,2 ppm and 232 ppb) were 6,5-7,8 folds higher than in Central Poland (2,6 ppm, 2,8 ppm, and 31 ppb, respectively).

Relationships between FHB indexes, FDK and accumulated mycotoxins in Central Poland were significant, except FHBi vs ZEA. Relationships between FHB indexes, FDK and accumulated mycotoxins in Western Poland were significant, except FHBi vs DON and NIV.

Some breeding lines showed lower FHBi, FDK and mycotoxin concentration than resistant variety "Tonacja" used as a control in both locations. In some cases breeding lines revealed lower FHB index, FDK or mycotoxin concentration than control resistant variety. Results showed potential for further improvement of resistance of winter wheat in Polish conditions.

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Keywords: breeding line, *Fusarium*, mycotoxin, deoxynivalenol, resistance, wheat

P79

Colonization of wheat rachides with *Fusarium culmorum* and *Fusarium graminearum*: species identity, fungal biomass and trichothecene chemotypeSara Asensio Manrubia¹, Christoph Brandfaß², Petr Karlovsky^{2*}¹Faculty of Biology, University of Barcelona (UB), Barcelona, Spain; ²Molecular Phytopathology and Mycotoxin Research, University of Göttingen, Göttingen, Germany

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Wheat ears afflicted with Fusarium head blight (FHB) were collected throughout Germany in 2003. Total DNA was extracted and the colonization by *F. graminearum* and *F. culmorum* was estimated by quantifying fungal DNA using species-specific qPCR assays (Brandfass C, Karlovsky P (2008) Upscaled CTAB-Based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *F. graminearum* DNA in plant material with reduced sampling error. Int J Mol Sci 9:2306). Essentially all rachides contained *F. graminearum*, *F. culmorum* or both, indicating that these two species were the causal agents of FHB in Germany ten years ago. *F. graminearum* turned out to be the major species across the entire country; a gradient from *F. culmorum* dominating in the North to *F. graminearum* dominating in the South, which was reported from Europe in the past decades, was not observed. We suggest that adaptation of *F. graminearum* to lower temperatures and/or extension of the maize cultivation to the northern parts of the country facilitating by breeding for cold tolerance accounts for the shift in the species distribution. Trichothecene chemotypes of the strains colonizing rachides was determined using a combination of several published PCR assays. A patchy distribution of chemotypes was found with chemotypes producing 3-ADON and 15-ADON exclusively found in small areas. NIV producers were rarely identified. These data were obtained on historical material that was preserved at -20°C. We intend to compare these results with the current situation in order to reveal mid-term changes in the species structure of *Fusarium* species responsible for FHB and of their trichothecene chemotype. An orientative map for *F. graminearum* and *F. culmorum* distribution in Germany as well as the major groups of trichothecenes produced on it have been made.

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