MycoTWIN - MycoKey

2021 INTERNATIONAL CONFERENCE

Integrated and innovative key actions for mycotoxin management in the food and feed chain

BARI, 9-12 November 2021

https://mycotwinbari2021.mycokey.eu/

BOOK of ABSTRACTS

ISBN: 978-88-8080-224-2 - **DOI:** https://doi.org/10.48257/ACBA01

ISM International Society for Mycotoxicology
research on mycotoxin and toxigenic fungi

Published by

Institute of Sciences of Food Production-National Research Council (CNR-ISPA)

Technical editors
Domenico GENCHI, Martina LOI, Dalia LEVITA, Alessandra VILLANI
Institute of Sciences of Food Production, (CNR-ISPA), Italy

Digital book by
Domenico GENCHI
Institute of Sciences of Food Production, (CNR-ISPA), Italy

ISBN digital version: 978-88-8080-221-1
ISBN printed version: 978-88-8080-224-2

DOI: https://doi.org/10.48257/ACBA01

Session **10 November 2021**

MycoTWIN-MycoKey 2021 OPENING

S1 – Biodiversity & Toxigenic Fungi Monitoring Chair

S2 – Challenges in Mycotoxin Analysis in food and feed

Session **10 November 2021**

S3 – Prevention of mycotoxigenic fungi in the field

Session **10 November 2021**

S4 – Food and feed Remediation, Intervention & Processing

Session **10 November 2021**

MAP1 - Human and Animal Health & Toxicology

S5 - Functional Genomics of Toxigenic Fungi

S6 – Control of mycotoxigenic fungi and mycotoxins in the field

Session **11 November 2021**

Voices from Mycotoxin Societies in the World

Session **12 November 2021**

S7 – Modelling and ICT solutions

MAP2 - Mycotoxin Control in Animal Feed

Session **12 November 2021**

Global impact of mycotoxins

Session

10 November 2021

INVESTIGATING HUMAN MYCOTOXIN EXPOSURE THROUGH UNITING LARGE-SCALE EPIDEMIOLOGICAL & MECHANISTIC POLY-OMIC DESIGNS

M. DE BOEVRE[1,2]
[1]*Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Ghent, Belgium;*
[2]*Cancer Research Institute Ghent, Ghent, Belgium;*
marthe.deboevre@ugent.be

"*Without good data, we are flying blind: if you can't see it, you can't solve it*" - Kofi Annan

Over the course of a human lifetime, dietary exposure to mycotoxins may be considered unavoidable. Mycotoxins have been suggested to contribute to a diversity of adverse health effects in humans, including carcinogenesis. Chronic low-dose multi-mycotoxin exposure was recently hypothesized by our research consortium to be associated with an increased risk of developing human hepatocellular and colorectal cancer. During this presentation focus is set to generate newly hypotheses-driven insights into the role of multiple mycotoxin exposure in the etiology of human carcinomas. To understand these potential effects on human health, the behavior of these mycotoxins in the human body is determined through both *in vitro* as human *in vivo* intervention studies by identifying and validating biomarkers of exposure and effect. The impact of mycotoxins on cancer development will be further investigated by measuring multiple mycotoxin biomarkers in biological samples in both healthy individuals as cancer patients in both Europe and Africa. Within the framework of the ITN UGent MYTOX-SOUTH® and the strong collaboration with a.o. WHO-IARC, access is granted to large epidemiological cohorts in both regions. The necessity of highly-powered epidemiological cohorts in mycotoxin & exposome research will be exploited by presenting the concept 'GLORIA - the Ghent Cohort'.

to Index

Session 1

Biodiversity & Toxigenic Fungi Monitoring Chair

MYCOTOXIN ACTIVITY IN THE FEED THE FUTURE LAB FOR REDUCTION OF POST-HARVEST LOSSES IN AFGHANISTAN, NEPAL AND HONDURAS

LESLIE J.F.
Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA
jfl@ksu.edu

The U.S Agency for International Development (USAID) sponsors a number of research efforts that pair US universities with partner universities and research organizations in a selected set of developing countries worldwide. Included in these Feed the Future Innovation Labs is the FTFIL for the Reduction of Post-harvest Losses. This lab has a major goal quantifying and reducing mycotoxin losses, especially aflatoxin, in stored food and feed. The lab promotes low cost moisture meters, portable grain dryers and hermetic storage bags to encourage high quality storage and to reduce increases in mycotoxin contamination during storage. In Afghanistan, nuts, raisins and wheat were evaluated for contamination, with aflatoxins in pistachios and ochratoxin A in raisins both present at high enough levels to seriously reduce the value, safety and international marketability of these valuable exports. Wheat in Afghanistan was virtually free of T-2 toxin, but often was contaminated with ergot. In Nepal, maize, peanuts, peppers and rice were evaluated, while in Honduras maize was the focus of study. Maize was contaminated with fumonisins at virtually every location, with aflatoxin present at a lower frequency but occasionally very high level. Peanuts could be contaminated with very high levels of aflatoxin, with peppers contaminated at a significant level, and rice essentially free of aflatoxin. The projects sought to sensitize all three governments to the significance of mycotoxin contamination in their domestic crops and to the potential economic and public health risks these compounds pose to domestic food and feed supplies and potential agricultural exports.

CLIMATE CHANGE ABIOTIC FACTORS AFFECT COLONISATION, BIOSYNTHETIC GENE EXPRESSION AND AFLATOXIN B_1 PRODUCTION BY *ASPERGILLUS FLAVUS* STRAINS IN RAW PISTACHIO NUTS

BAAZEEM, A.[1,2], RODRIGUEZ, A.[1,3], MEDINA, A.[1], MAGAN, N[1].
[1]*Applied Mycology Group, Environment and AgriFood Theme, Cranmfield University, Cranfield. Beds. MK43 oAL, U.K.* [2]*Dept. of Biology, College of Science, Taif University, Saudi Arabia,* [3]*Dept. of Animal Science and Food Production, University of Extramadura, Badajoz, Spain.*
n.magan@cranfield.ac.uk

Pistachio nuts are an important economic tree nut crop which is used directly or processed for many food-related activities. They are colonized by mycotoxigenic spoilage fungi, especially *Aspergillus flavus*, resulting in contamination with aflatoxins (AFs), especially aflatoxin B_1 (AFB_1). The prevailing climate in which these crops are grown changes as temperature and atmospheric CO_2 levels increase, and episodes of extreme wet/dry cycles occur due to human industrial activity. The objectives of this study were to evaluate the effect of interacting Climate Change (CC)-related abiotic factors of temperature (35 vs. 37°C), CO_2 (400 vs. 1000 ppm) and water stress (0.98–0.93 water activity, a_w) on (a) colonization, (b) *aflD* and *aflR* biosynthetic gene expression and (c) AFB_1 production by strains *A. flavus* (AB3, AB10) when colonizing raw pistachio nuts. In addition the effect of acclimatization for 5 generations was examined for the resilience and effects on AFB_1 production on pistachio nuts. The *A. flavus* strains were very resilient in terms of colonization of pistachio nuts with no significant difference when exposed to the interacting three-way climate-related abiotic factors. The relative expression of the structural *aflD* gene involved in AFB_1 biosynthesis was decreased or only slightly increased, relative to the control conditions at elevated CO, regardless of the a_w level examined. For the regulatory *aflR* gene expression, there was a significant ($p < 0.05$) increase in 1000 ppm CO_2 and 37°C for both strains, especially at 0.95 a_w. There was a significant ($p < 0.05$) stimulation of AFB_1 production at 35°C and 1000 ppm CO_2 for both strains, especially at 0.98 a_w. At 37 °C, AFB_1 production was either decreased, or remained similar depending on the strain when exposed to 1000 ppm CO_2. Acclimatized strains of *A. flavus* (5 generations) showed changes in colonization patterns and some stimulation in AFB_1 production in pistachio nuts. This suggests that *A. flavus* strains are very resilient to climate change factors, with differential effects on AFB_1 production, that may be strain dependent. This will impact on the relative toxin risks during processing of this tree nut under future climate-related abiotic factors.

POPULATION GENETIC DIVERSITY OF PATHOGENS OF FUSARIUM CROWN ROT AND ITS POTENTIAL MYCOTOXIN CONTAMINATION ON WHEAT

ZHANG H.
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
zhanghao@caas.cn

Fusarium crown rot (FCR) caused by Fusarium spp. has been prevalent in China in recent years, and its damage is increasing. FCR causes yield loss of wheat, and also lead to potential threat of mycotoxin contamination. This research is an investigation of the composition of Fusarium species in the major wheat area in China. The genetic diversity in the dominant species was studied, and the population genetic relationship of Fusarium graminearum species complex (FGSC) derived from FCR and Fusarium head blight (FHB) was analyzed. In order to evaluate the risk of mycotoxin contamination caused by FCR, the contents and the distribution of mycotoxin in plants and the mycotoxin contamination of diseased wheat plants after natural infection of FCR in the field were determined in Northern China.

BIODIVERSITY OF TOXIGENIC FUNGI IN THE FOOD CHAIN AND THE CO-OCCURRENCE OF MYCOTOXINS.

VENÂNCIO A.[1*], VALE DIAS T.[1], GUIMARÃES A.[1], CALADO T.[1] and RODRIGUES P.[2].

[1]*CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;* [2]*Centro de Investigação de Montanha (CIMO); Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*

avenan@deb.uminho.pt

Filamentous fungi are ubiquitous in Nature and may be found in any food crop, either in the field or during storage. With the exponential growth of the population, there is a worldwide challenge to reduce food losses. Food contamination by fungi causes great economic costs and several health threats due to the toxicity and pathogenicity of some species. The biodiversity of this community, and the dynamics of the water activity, temperature and availability of carbon sources evolution, during the growing season, as well as in storage, determines the competitiveness of each species against other co-occurring fungal species.

Some of these fungi are capable of producing a wide range of secondary metabolites – mycotoxins, which may accumulate in food chain, be resilient during food processing, and persist in the final food product. Contamination of food products with fungi is frequent, affecting food security and food safety. The ability of the different fungal species to compete under available conditions will influence the cocktail of mycotoxin that may occur.

The study of the fungal biodiversity have been mainly carried out by surveying culturable strains (culture-dependent approaches), but recently metagenomics approaches have been used, enabling the possibility of spotting mycotoxigenic fungal strains, that are not easy to detect and isolate by conventional means.

In this presentation, the biodiversity of fungal species in the food chain will be discussed, under a perspective of the co-occurrence of mycotoxins.

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 (CEB) and UID/AGR/00690/2019 (CIMO) units. We also thank the FCT for the Ph.D. scholarship given to Teresa Vale Dias (2020.05849.BD).

DIVERSITY OF FUSARIUM HEAD BLIGHT PATHOGENS IN BRAZIL AND A GLOBAL DATABASE OF *FUSARIUM GRAMINEARUM* SPECIES COMPLEX

DEL PONTE E.M.
Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa MG 36570-900 Brazil
delponte@ufv.br

Species within the former *Fusarium graminearum* species complex (FGSC), together with a few others of the new *Fusarium sambucinum* species complex (that incorporated FGSC), are the cause of many floral diseases that affect small grain cereals including wheat, barley and oats. In Brazil, surveys have been conducted in my laboratory during the last 15 years to unveil the complex of mycotoxigenic species associated with Fusarium head blight (FHB) of wheat, among other pathosystems. The analysis of more than a thousand isolates revealed a unique large diversity of FHB pathogens in Brazil compared with most other wheat production regions worldwide. The two most significant species identified have been *F. graminearum* and *F. meridionale*, but recent works have shown the emergence of other species including *F. poae* and *F. avenaceum*. The temporal and spatial variations in the frequency of these species will be presented in the first part of my talk. Finally, I will present the results of a collaborative effort to collect and analyse bibliographic FGSC data from over a hundred publications from 2000 to 2021, together with the construction and analysis of a global FGSC database. Spatial and temporal patterns of the FGSC distribution can be accessed via a publicly available web-based application, allowing for searches, summarization and mapping of strains according to several criteria including article, country, host, species and trichothecene genotype. The database is accessible at https://fgsc.netlify.app/.

MYCOSUGAR: A POLYPHASIC APPROACH TO IDENTIFY TOXIGENIC FUNGI AND QUANTIFY MYCOTOXINS IN SUGARCANE AND DERIVED PRODUCTS

ABDALLAH MF.[1,2*], BEREKET C.[1], LUST L.[1], REYNAERT L.[1], PAVICICH M.A.[1,3], ANELLI P.[2,4], LANDSCHOOT S.[2], V. KAGOT[1,5], L. MATUMBA[6], B. BEKAERT[2], S. OKOTH[5], A. SUSCA[4], M. DE BOEVRE[1], G. HAESAERT[2], J. HOUBRAKEN[7], K. AUDENAERT[2], S. DE SAEGER[1].

[1]Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium; [2]Laboratory of Applied Mycology and Phenomics, Ghent University, Belgium; [3]Universidad de Buenos Aires, Laboratorio de Microbiología de Alimentos, Buenos Aires, Argentina; [4]Institute of Sciences of Food Production, ISPA-CNR, Bari, Italy; [5]Centre for Biotechnology and Bioinformatics, University of Nairobi, Kenya; [6]Department of Agricultural Research Services, Chitedze Research Station, Malawi; [7]Westerdijk Fungal Biodiversity Institute, Department of Applied and Industrial Mycology, Utrecht,The Netherlands

mohamed.fathi@ugent.be

The aim of MYCOSUGAR was to quantify mycotoxins in sugarcane grass and its by-products; to identify the toxigenic fungi and their secondary metabolites; to assess the risk in human due to exposure to mycotoxins present in sugarcane juice. For this purpose, two sampling rounds were done. The first set comprised samples from sugarcane grass (n= 135) and jaggery (n=50) originating from Malawi and Kenya. The second set of the samples were sugarcane juice samples (n=89) collected from Egypt in two different seasons. Furthermore, an LC-MS/MS method was validated to detect the (co-)occurrence of 13 mycotoxins. Based on the obtained occurrence data, a risk assessment of mycotoxins using probabilistic and deterministic approaches including various scenarios for adult male and female Egyptian juice consumers was performed. Briefly, aflatoxin B_1 and fumonisin B_1 co-occurred in sugarcane juice, grass, and jaggery. For the samples originating from Egypt, a remarkable difference in levels of exposure to mycotoxins between the two seasons and between male and female juice consumers was observed.

Further, a polyphasic approach was applied by isolating toxigenic fungi from the sugarcane samples and the derived products to identify the isolated fungal species. Both a morphological and molecular identification of the mycotoxigenic fungi was performed. A dereplication strategy was also applied to investigate the diversity of the secondary metabolites using UHPLC–TOF-MS. Details on the results and future perspectives of mycotoxins and their producing fungi in sugarcane as an important industrial crop will be presented during the conference.

This research was partially supported by "a Global Minds Grant 2019", Ghent University to the first author.

BIODIVERSITY OF ENDOPHYTIC *FUSARIUM* SPECIES IN POTATO PLANTS FROM TWO REGIONS OF IRAN

ALIJANI N.[1*], MASIELLO M.[2], SOMMA S.[2], SAREMI H.[1], JAVAN NIKKHAH M.[1], PETRINI O.[3], MORETTI A.[2], C. ALTOMARE[2].

[1]*Department of Plant Protection, Colledge of Agriculture and Natural resource, University of Tehran, Karaj, Iran.* [2]*CNR-ISPA, Bari, Italy.* [3]*Institute of microbiology, Department of Environment Construction and Design, University of Applied Sciences and Arts of Southern Switzerland, Bellinzona, Switzerland.*

Alijani.nasim@ut.ac.ir, Alijani.nassim@gmail.com

Endophytic fungi live inside healthy plant tissues without causing any apparent symptoms or damage to their host. Endophytic associations may bring benefits to their hosts, such as enhancing host plant defense, limiting pathogens damage, modulating seedling development by producing plant signal molecules and growth regulators or nutrients, and inducing tolerance to biotic and abiotic stresses. In this study, endophytic fungi of potatoes were isolated from samples collected during summer and winter 2018 from two Iranian provinces, *viz.* Ardebil and south Kerman. Eighty samples were collected from mature and healthy potato plants. Surface sterilization was carried out by immersion of the samples in 70% ethanol for two minutes, 5% sodium hypochlorite for 5 minutes, and then washed three times in distilled water. Almost 1000 isolates belonging to approximately 30 genera were obtained, with 300 isolates belonging to *Fusarium*. Based on their origin (potato farm, plant organ) and their morphological features, 100 isolates were selected for molecular identification. The translslation elongation factor 1-alpha genomic region was amplified, and a phylogenetic analysis of the results was carried out using MEGA 6. The main *Fusarium* species identified were *F. nygamai*, a species belonging to the *Fusarium fujikuroi* species complex (FFSC), species of the *F. oxysporum* species complex (FOSC), and species of the *F. solani* species complex (FSSC). At a lesser extent, also other species belonging to FFSC, *F. incarnatum-equiseti*, *F. redolens* and *F. sambucinum* species complexes were identified. The amplification of other genomic regions such as beta-tubulin and LSU is currently ongoing, with the aim to complete the phylogenetic analyses of these isolates.

N.A fellowship at CNR-ISPA, Bari is supported financially by the Iran National Science Foundation (INSF), project number: 98015699.

POLYPHASIC CHARACTERIZATION OF *ASPERGILLUS FLAVUS* AND *A. PARASITICUS* ISOLATED FROM ETHIOPIAN PEANUT-SOIL

CERVINI C.[1], VERHEECKE-VAESSEN C.[1], MOHAMMED C.[2], MAGAN N.[1], MEDINA A.[1]

[1]*Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, UK;*
[2]*Haramaya University, School of Plant Sciences, P.O. Box138, Haramaya, Ethiopia.*
Carla.Cervini@cranfield.ac.uk

Peanut production in Ethiopia is important economically but has significant problems because of the prevalence of high contamination levels with aflatoxins which represents a significant health risk for use in both food and feed chains. Little is known about the fungal populations and their mycotoxin production capacity in Ethiopian peanuts. The aim of this study was to identify and characterize *A. flavus* and *A. parasiticus* isolates from rural Ethiopian peanut production areas in both the plant and soil. A polyphasic approach, consisting of morphological (macro- and microscopical), molecular analyses and mycotoxin production profiles were used to characterize twenty-four isolates.

Morphological identification was performed on Malt Extract Agar (MEA) and Czapek Yeast Extract Agar (CYA) at 25, 30, 37 and 42°C after 7 days of incubation. Two morphotypes were distinguished: *A. flavus* (n=19) and *A. parasiticus* (n=5) showing green and dark green colony colours on MEA, respectively. Dark sclerotia were observed after 14 days on MEA30 in 60% of the *A. flavus* isolates with higher prevalence of L-type (> 400 µm). All the isolates were positive (bright orange colour) for Aspergillic acid on the selective medium for *Aspergillus flavus* and *A. parasiticus* agar (AFPA) after 72 hours. Mycotoxin analyses, performed with LC-MS/MS qTRAP, revealed three chemotypes: (i) (n=23), (ii) aflatoxins B (AFB) aflatoxin G (AFG) (n=5), (iii) atoxigenic (n=1).

Molecular analysis based on sequencing of calmodulin gene (cmd5-cmd6) and Internal Transcribed Spacer (ITS1-ITS4) regions supported the morphological and secondary metabolite findings showing, on average, more than 98% matching identity for *A. flavus* (n=23) and *A. parasiticus* (n=5) strains. This is the first detailed study characterising *A. flavus* and *A. parasiticus* strains isolated from Ethiopian peanut-soil ecosystems using a polyphasic approach. The accurate identification of these species provides new useful data to estimate the aflatoxigenic risk in the peanut chain and will help in the development of effective control strategies in the Ethiopian peanut production chain for both food and feed use.

This research was part of the project NutriNuts (105663) funded by Innovate UK.

FUSARIUM CHLAMYDOSPORUM SPECIES COMPLEX ASSOCIATED WITH BRAZILIAN RICE: SPECIES DIVERSITY, TOXIGENIC POTENTIAL AND DETECTION OF THE GENE CLUSTER FOR THE PRODUCTION OF TRICHOTHECENES

COSTA M.M.[1], GOMES L.B.[1], VILLANI A.[2], DEL PONTE E.M.[3]; PFENNING L.H.[1], LOGRIECO A.F.[2], MORETTI A.[2], SUSCA A.[2]

[1]Plant Pathology Department, Universidade Federal de Lavras, Lavras, MG, Brazil; [2]Institute of Sciences of Food Production – ISPA/CNR, Bari, Italy. [3]Plant Pathology Department, Universidade Federal de Viçosa, Viçosa MG, Brazil.

costa.marileide92@gmail.com

Seventy seven strains of the *Fusarium chlamydosporum* species complex (FCSC), isolated from seeds and grains of rice sampled in the main production areas in Brazil, were identified as member of complex based on observation of morphological markers, such as the presence of chlamydospores and formation of conidiophores in the aerial mycelium with a typical ramification. Then, the strain identity was confirmed by multilocus sequence analysis of partial sequences of t*ef-1α, rpb2* and *caM* genes. Phylogenetic analyses of t*ef-1α, rpb2* and *caM* identified three known phylogenetic lineages belonging to FCSC: *F. chlamydosporum* (n=56), *F. atrovinosum* (n=8), and *F. spinosum* (n=13). The potential to produce mycotoxins was also evaluated for all strains: production of beauvericin, enniatin, T2-toxin, HT2-toxin, nivalenol, deoxynivalenol, and its acetylated forms 3-acetildeoxynivalenol (3-ADON) and 15-acetildeoxynivalenol (15-ADON) was tested in pure cultures. Isolates of all three lineages produced detectable levels of beauvericin, enniatin, T2-toxin, HT2-toxin and NIV *in vitro*. There was no production of detectable levels of DON, 3-ADON and 15-ADON. Furthermore, the analysis for the occurrence of 2 genes (*tri5* and *tri1*) involved in trichothecenes production using PCR approach showed their presence in two out of ten analyzed strains. In conclusion, we report here the occurrence on rice of three species belonging to FCSC, and for the first time we detect trichothecene production by FCSC species. Further analyses are in progress to confirm this preliminary evidence.

GENOMIC COMPARATIVE ANALYSIS AND GENOME EDITING REVEALED THE INVOLVEMENT OF A CYCLASE GENE IN THE BIOSYNTHESIS OF OCHRATOXIN A.

FERRARA M.[1*], GALLO A.[2], CERVINI C.[3], GAMBACORTA L.[1], SOLFRIZZO M.[1], BAKER S.E.[4,5], PERRONE G.[1]

[1]Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Bari, Italy; [2]Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Lecce, Italy; [3]Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, UK; [4]Functional and Systems Biology Group, Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, WA, United States of America; [5]DOE Joint Bioenergy Institute, Emeryville, CA, United States of America

massimo.ferrara@ispa.cnr.it

The widespread use of Next-Generation Sequencing (NGS) for fungal genome sequencing has led to identification of SM clusters for known metabolites as well as a significant number of novel predicted SM gene clusters. Fungal genome sequencing has great utility for identification of secondary metabolites gene clusters for known as well as novel compounds. Ochratoxin A (OTA) is a well-known mycotoxin with wide distribution on food and feed. A comparative analysis of gene cluster structure in 19 *Aspergillus* and 2 *Penicillium* OTA producers has revealed a high synteny across these species in OTA cluster, encoding five structural genes: *otaA*, *otaB*, *otaC*, *otaR1*, and *otaD*. Furthermore, we identify a previously undescribed additional gene. The new *otaY* gene is located between the *otaA* and *otaB* genes and encodes a protein with predicted SnoaL domain. These snoaL-domain containing proteins have been shown to catalyze ring closure steps in the biosynthesis of polyketide antibiotics produced in *Streptomyces*. Gene expression analysis has demonstrated an up-regulation of the cyclase gene in *A. carbonarius* under OTA permissive conditions, consistent with the expression trends of the other OTA cluster genes. The role of in OTA biosynthesis has been demonstrated by CRISPR/Cas9 complete gene deletion. The presented results reveal for the first time the involvement of a cyclase gene in OTA biosynthetic pathway and redefine the structure of the OTA core cluster, consisting in six genes.

ASPERGILLUS VINACEUS, A NEW SPECIES IN *ASPERGILLUS* SUBGENUS *CIRCUMDATI* SECTION *NIGRI*

FUNGARO M.H.P.[1,2], DA SILVA J.J.[1,2], FERRANTI L.S.[1], MASSI F.P.[1], TANIWAKI M.H.[2], PUEL O.[3], LORBER S.[3], FRISVAD J.C.[4], IAMANAKA B.T.[2]

[1]Universidade Estadual de Londrina, Londrina, Paraná, Brazil; [2]Food Technology Institute, ITAL, Campinas, São Paulo, Brazil; [3]Toxalim (Research Centre in Food Toxicology), INRAE, ENVT, INP-Purpan, Toulouse, France. [4]Technical University of Denmark, Lyngby, Denmark.

mariafungaro@gmail.com.

We investigated the genetic diversity of the *Aspergillus niger* clade using calmodulin gene (*CaM)* sequences of approximately 700 accessions belonging to this clade. Eight haplotypes were identified as *A. niger* (*n*= 247), and 17 were identified as *Aspergillus welwitschiae* (*n*= 403). However, six haplotypes, representing 45 strains, were not resolved using *CaM* sequences. To elucidate the taxonomic position of these haplotypes two other *loci*, part of the beta-tubulin gene (*BenA*) and part of the RNA polymerase II gene (*RPB2*), were sequenced. Using Genealogical Concordance Phylogenetic Species Recognition (GCPSR) approach, two new Phylogenetic Species (PS2 and PS3) were recognized. The PS3 strains were found morphologically distinct in relation to *A. niger* and *A. welwitschiae* strains. The strains of the Phylogenetic Species PS3, showed reduced growth on MEA medium, when grown on CYA (25 °C), white mycelial area staining with very sparse sporulation and sclerotia production with ovoid shape, color varying from white to cream, measuring on average 1,200 ± 300 µm on MEA and CYA. Based on secondary metabolites, the PS3 strains differed chemically from *A. niger*, *A. welwitschiae*, *Aspergillus tubingensis, Aspergillus neoniger, Aspergillus eucalypticola* and *Aspergillus luchuensis*. Concluding, when the results of Genealogical Concordance Phylogenetic Species Recognition and phenotypical evidences were jointly considered, our study revealed a new species in *Aspergillus* subgenus *Circumdati* section *Nigri*. Because all strains of this species were isolated from grape samples (*Vitis labrusca* L.), it will be described as *Aspergillus vinaceus*.

This research was financially supported by CNPQ (Project 311240/2019-4 and 303732/2018-0) and Fundação de Amparo a Pesquisa do Estado de São Paulo (Project 2019/06032-8, 2013/05414-8).

DETECTION OF *FUSARIUM GLOBOSUM* IN SMALL GRAIN CEREALS ON THE URAL AND WESTERN SIBERIA TERRITORY

GAGKAEVA T.1*, ORINA A.1, GAVRILOVA O.1, LEBEDIN Y.2

[1]All-Russian Institute of Plant Protection (VIZR), 196608, St.-Petersburg, Pushkin, Russia; [2]XEMA Company Limited, 105264, Moscow, Russia

t.gagkaeva@yahoo.com

Members of the *Fusarium fujikuroi* species complex (FFSC) are among the most common fungi reported on crops worldwide. On Russian territory, several species of FFSC such as *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, and *F. fujikuroi* have been detected in different crops including cereals. In 2017-2018, 103 grain samples from the Ural and Western Siberia were analyzed. The *Fusarium globosum* isolates were revealed in cereals grown at a distance of at least 1500 km: in barley grain from Novosibirsk region, and in bread and durum wheat grain from the Altai Krai and Chelyabinsk region (Gagkaeva et al., 2019). The morphological identification of the strains was confirmed by DNA sequencing of the translation elongation factor 1α gene. There were no significant differences between *F. globosum* and other species from FFSC in the temperature optimum of growth, but the range of favorable temperatures for *F. proliferatum* and *F. verticillioides* was significantly wider than for *F. globosum* strains. The isolated *F. globosum* strains were low pathogenic to wheat leaves. The presence of this species in Russia was reported for the first time (Gagkaeva et al., 2019). Previously, *F. globosum* was isolated from maize in Southern Africa (Rheeder et al., 1996) and from wheat culms in subtropical Japan (Aoki, Nirenberg, 1998). *F. globosum* is deeply nested within the Asian clade in the molecular phylogeny (O'Donnell et al., 1998, Proctor et al., 2013). Our findings confirm that *F. globosum* is naturally occurring species on Asian territory. y.

This study was financially supported by the Russian Science Foundation (project No. 19-76-30005).

GENETIC POLYMORPHISMS ASSOCIATED TO SDHI FUNGICIDES RESISTANCE IN SELECTED ASPERGILLUS FLAVUS STRAINS AND RELATION WITH AFLATOXIN PRODUCTION

MASIELLO M., SOMMA S., HAIDUKOWSKI M.,
LOGRIECO A.F., MORETTI A.
Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via Amendola 122/O, 70126, Bari, Italy.
mario.masiello@ispa.cnr.it

Aspergillus flavus is a harmful fungal species able to colonize several crops, in both pre- and post-harvest conditions, and to synthesize aflatoxin B1, a mycotoxin classified as group 1 (human carcinogenic) by the International Agency for Research on Cancer. Several approaches have been proposed to control *A. flavus* development and aflatoxin B1 production. The Succinate Dehydrogenase Inhibitor (SDHI) fungicide boscalid is effective to control *A. flavus* growth and aflatoxin contamination. However, this compound is classified as medium-high risk fungicide.
In this paper, we selected laboratory *A. flavus* boscalid resistant strains and investigated the molecular mechanism associated to resistance. Specific primer pairs were designed to amplify the *SdhB*, *SdhC* and *SdhD* genes. By amino acidic sequence analysis, two point mutations, Tyrosine replacing Histidine at codon 249 of *SdhB* and Arginine replacing Glycine at codon 91 of *SdhC*, were identified. The effect of SDHI boscalid and isopyrazam on mycelial growth and conidial germination was evaluated. Both resistant genotypes showed high resistance (MIC and EC50 > 1000 mg/L) to boscalid. A positive cross-resistance was found between boscalid and isopyrazam. Specific sub-lethal doses, 0.5 mg/L of boscalid and 0.01 mg/L of isopyrazam, have been associated to a strong depigmentation of colonies, and increasing of aflatoxin production.

GENETIC AND CHEMICAL ANALYSES OF *ALTERNARIA* AND *CURVULARIA* SPECIES ASSOCIATED TO LEAF SPOT DISEASE ON DATE PALM, IN SOUTH TUNISIA

RABAAOUI A.[1], MASIELLO M.[2*], SOMMA S.[2], DALL'ASTA C.[3], RIGHETTI L.[3], SUSCA A.[2], LOGRIECO A.F.[2], NAMSI A.[4], GDOURA R.[1], WERBROUCK S. P.O.[5], MORETTI A[2].

[1]Laboratory of Toxicology-Microbiology and Environmental Health, Department of Biology, University Sfax, Sfax, Tunisia; [2]National Research Council, Institute of Sciences of Food Production, Bari, Italy; [3]Department of Food and Drug, University of Parma, Parma; [4]Laboratoire de Phytopathologie, Centre Régional de Recherches en Agriculture Oasienne, Degache, Tunisia; [5]Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University,Ghent, Belgium

mario.masiello@ispa.cnr.it

Date palm (*Phoenix dactylifera* L.), is widely cultivated across North Africa. In Tunisia, with about 300 thousand tons of fruits produced per year, and occupies a strategic place in the socio-economic stability of semiarid and arid regions. Several biotic and abiotic factors can compromise date palm cultivation. A wide range of fungal pathogens have been associated to date palm plants showing symptoms of leaf spots. However, *Alternaria* species are reported as the most frequent pathogens. Samples of symptomatic plant parts of the common *Deglet Nour* variety were randomly collected in six localities, in Tunisia. Among the fungal strains, we have selected 50 strains, based on morphological features, that were shown to be *Alternaria* (45 strains) and *Curvularia* (5 strains). A polyphasic approach, based on morphological characterization, phylogenetic studies and analyses of mycotoxin profile, was carried out for each strain, in order to provide a correct identification at species level. In addition, the pathogenicity of representative strains for each species was tested on date palm plantlets.

Sequencing of *allergen-alt-1a*, *glyceraldeyde3-phosphate dehydrogenase* and *calmodulin* genes allowed us to group 35 strains, identified as *A. arborescens*, *A. tenuissima* and *A. alternata* species, in *Alternaria* Section, and 10 strains identified as *A. consortialis* in *Ulocladioides* Section. Based on combined ITS, GPD and EF-1α genomic regions sequencing analyses, all 5 *Curvularia* strains were identified as *C. spicifera*. The capability to produce *in vitro* alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tenuazonic acid (TA), tentoxn (TEN) was analyzed. All *A. consortialis* strains did not produced any mycotoxin, whereas more than 80 % of the strains, included in *Alternaria* section, produced very variable amounts multiple mycotoxins such as AOH, AME, TEN and TA. In addition, 40 % of the strains *Alternaria* section produced also ALT. Finally, all *Alternaria* and *Curvularia* strains, tested on date palm plantlets, were pathogenic, complying Koch's postulates.

This work reports a comprehensive multidisciplinary study of fungal pathogens associated to leaf spot disease on date palm, that regards both phytopathological and food safety issues.

EFFECT OF STEADY STATE OR FLUCTUATING TEMPERATURE REGIMENS ON THE PRODUCTION OF ZEARALENONE BY TWO STRAINS OF *FUSARIUM GRAMINEARUM*

JAKOVČEVIĆ Z.1, FARKAŠ H.1, RAJ J.*1, MAGAN N.2, BOŠNJAK-NEUMÜLLER J.1, VASILJEVIĆ M.1

[1]PATENT CO, DOO., Vlade Ćetkovića 1A, 24 211, Mišićevo, Serbia.;[2]Applied Mycology Group, Vincent Building, Cranfield University, Cranfield, Bedford, MK43 0AL, UK.

jog.raj@patent-co.com

In this study, the effect of steady state or fluctuating temperatures on the production of zearalenone by two strains of *Fusarium graminearum* was examined. The strains were cultivated on ground corn (maize) substrate for 28 days. The temperatures examined were (a) steady state cultivation at 15 or 25°C for 28 days and (b) fluctuating temperatures between 25°C and then lowering to 15°C. At the end of the incubation period, the cultures were air-dried at 105°C and the zearalenone production quantified using LC-MS/MS. The optimum production level of zearalenone was obtained in substrate incubated at 25°C for 14 days and then on 15°C for 14 days. Overall, the maximum mean zearalenone production was obtained when incubation was done for 28 days at 25°C. These results are discussed in context of the production of mycotoxins for use in evaluating efficacy of different binders for the feed chain.

FUSARIUM FUJIKUROI SPECIES COMPLEX ASSOCIATED WITH RICE, MAIZE AND SOYBEAN FROM JIANGSU PROVINCE, CHINA: PHYLOGENETIC, PATHOGENIC AND TOXIGENIC ANALYSIS

QIU J.[1], LU Y.[2], HE D.[1], LEE Y.W.[3], XU J.[1], SHI J.[1*]

[1]Jiangsu Key Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base, Ministry of Science and Technology/ Key Laboratory for Control Technology and Standard for Agro-product Safety and Quality, Ministry of Agriculture and Rural Affairs/ Collaborative Innovation Center for Modern Grain Circulation and Safety/ Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China; [2]College of Marine Life and Fisheries, Jiangsu Ocean University, Lianyungang 222005, China; [3]Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea.

shiji@jaas.ac.cn

A total of 237 candidate strains were isolated from rice, maize and soybean samples in Jiangsu Province, China. Species identification of the individual strain was accomplished by sequencing the translation elongation factor 1α gene (*TEF-1α*) and the fumonisin (FB) synthetic gene (*FUM1*). The distribution of *Fusarium* species among the different crops was observed. The maize seeds were dominated by *F. proliferatum* and *F. verticillioides*, while *F. fujikuroi* was the most frequently isolated species from rice and soybean samples. Additionally, phylogenetic analyses of these strains were performed, and the results suggested clear groups showing no obvious relationship with the origin source. The pathogenicity and toxigenicity of the FFSC species were studied. All the species reduced the rice seed germination rate and produced lesions on the maize leaves, with no significant differences. *F. fujikuroi* showed two distinct patterns of influence on the length of rice seedlings, which were correlated with FB and gibberellic acid synthesis. FBs were mainly produced by *F. verticillioides* and *F. proliferatum*. *F. proliferatum* and *F. fujikuroi* also produced moniliformin and beauvericin. The toxigenicity of *F. andiyazi* was extremely low. Further analysis indicated that the sequence variations in *TEF-1α* and the differences in the expression levels of the toxin synthesis genes were associated with the diversity of secondary metabolites in *F. fujikuroi* strains. These findings provide insight into the population-level characterization of the FFSC and might be helpful in the development of strategies for the management of diseases and mycotoxins.

This work was supported by the International Science & Technology Cooperation Program of China (2016YFE0112900) National Natural Science Foundation of China (31701748, 31772118).

IDENTIFICATION OF TOXIGENIC FUNGAL SPECIES ASSOCIATED WITH MAIZE EAR ROT: CALMODULIN AS SINGLE INFORMATIVE GENE

SUSCA A.[1]*, VILLANI A.[1], MORETTI A.[1], STEA G.[2], A.F. LOGRIECO[1]
[1]*Institute of Sciences of Food Production, National Research Council (CNR), Italy.*
[2]*Institute for Biomedical Technologies, National Research Council (CNR), Italy*
antonella.susca@ispa.cnr.it

Accurate identification of fungi occurring on agrofood products is the key aspect of any prevention and pest management program, offering valuable information in leading crop health and food safety.
Fungal species misidentification can dramatically impact biodiversity assessment, ecological studies, management decisions, and, concerning toxigenic fungi, health risk assessment, since they can produce a wide range of toxic secondary metabolites, referred to as mycotoxins. This can be especially important with maize, since it is a valuable food resource globally. Since each toxigenic fungal species can have its own mycotoxin profile, a correct species identification, hereby attempted with universal DNA barcoding approach, could have a key role in mycotoxins prevention strategies. Currently, identification of single marker for fungi has not been achieved and the analysis of multiple genes is used, with the advantage of an accurate species identification and disadvantage of difficult setting up of PCR-based diagnostic assays.
In the present paper, we describe our strategy to set up DNA-based species identification of fungal species associated with maize ear rot, combining DNA barcoding approach and species-specific primers design for PCR based assays. We have (i) investigated the appropriate molecular marker for species identification, limited to mycobiota possibly occurring on maize, identifying calmodulin gene as single gene taxonomically informative; (ii) designed 17 set of primers for rapid identification of 14 *Fusarium*, 10 *Aspergillus*, 2 *Penicillium*, and 2 *Talaromyces* species or species groups, and finally (iii) tested specificity of the 17 set of primers, in combination with 3 additional set previously developed.

BIODIVERSITY OF ASPERGILLUS SECTION FLAVI AND A. SECTION NIGRI IN BRAZILIAN FOODSTUFFS

TANIWAKI M.H.[1], FUNGARO M.H.P.[1,2], FRISVAD J.C.[3], SILVA J.J.[2],MARTINS L.M.[1], KATSURAYAMA A.M.[1], FERRANTI L.S.[1], IAMANAKA B.T.[1]

[1] Food Technology Institute – ITAL, Campinas, SP, Brazil; [2]Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil, [3]Technical University of Denmark, Lyngby, Denmark

marta@ital.sp.gov.br

Brazil has a large biodiversity of fungi, including *Aspergillus* species. This group stands out for their high frequency in food, which is of concern as they are spoilage fungi, moreover, many *Aspergillus* species are potentially toxigenic. The aim of this study was to investigate the biodiversity of *Aspergillus* sections *Flavi* and *Nigri* in Brazilian foodstuffs: peanuts, rice, sugarcane and grapes isolated along the processing chain. The samples were plated onto DG18 agar and incubated at 25ºC. The fungi were identified using a polyphasic approach. In peanuts, four species of *A.* section *Flavi* were isolated: *A. caelatus, A. flavus, A. parasiticus* and *A. tamarii*. In rice, five species belonging to A. section *Flavi* were found: *A. flavus, A. caelatus, A. novoparasiticus, A. arachidicola* and *A. pseudocaelatus.* The most common species in peanuts and rice was *A. flavus*, with 50% and 17% of the isolates able to produce aflatoxins, respectively. In sugarcane, *A. novoparasiticus* and *A. arachidicola* were the predominant species, all isolates were aflatoxin producers. In grapes, the prevalent species of *A*. section *Nigri* were: *A. japonicus* and *A. uvarum* (among uniseriate species) and *A. niger* (among biseriate species), other black aspergilli species were also present: *A. brunneoviolaceus, A. aculeatus, A. labruscus, A. carbonarius, A. welwitschiae* and *A. vadensis*. Only 3.2% of *A.* section *Nigri* isolates were OTA producers. Among the *A. niger* "aggregate" species 42.1% were FB_2 producers. Concluding, great diversity of *Aspergillus* species was found and a strong association of some species with their substrates of origin was observed.

This research was financially supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo Project 2011/ 10073-0, 2013/05414-8, 2014/07498-7, 2017/00824-4, 2018/25597-3, 2019/06032-8 and "Conselho Nacional de Desenvolvimento Científico e Tecnológico Grant 303732/2018-0, 311240/2019-4

PHYLOGENY AND MYCOTOXIN PROFILE OF *FUSARIUM* SPECIES ISOLATED FROM SUGARCANE IN SOUTHERN IRAN

TAVAKOL NOORABADI M.[1], MASIELLO M.[2], TAHERKHANI K.[3], ZARE R.[4], TORBATI M.[5], HAIDUKOWSKI M.[2], SOMMA S.[2], LOGRIECO A.F.[2], MORETTI A.[2], SUSCA A.[2*]

[1]Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Sari, Iran; [2]National Research Council, Institute of Sciences of Food Production, Bari, Italy; [3]Sugarcane Research and Training Institute for the Development of Industries in Khuzestan, Ahvaz, Iran; [4]Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization, Tehran, Iran; [5]Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

antonella.susca@ispa.cnr.it

Sugarcane is an important crop for agri-food, energy, and pharmaceutical industries. Among the pathogens that colonize sugarcane, *Fusarium* species are reason of serious concern for both their pathogenicity on plants and ability to produce harmful mycotoxins. We studied 104 *Fusarium* strains isolated from sugarcane in Southern Iran. Based on morphological and phylogenetic analyses, 98 strains belonged to *Fusarium fujikuroi* Species Complex (FFSC) and identified as *F. proliferatum* (68.5 %), *F. sacchari* (14.5%), *F. thapsinum* (2%) and *F. verticillioides* (1%). In addition, 14 FFSC strains, although phylogenetically closely related to *F. andiyazi*, could not be assigned to any known species, likely representing a new phylogenetic species. Three strains identified as *Fusarium incarnatum equiseti* Species Complex, two *F. solani* strains and a *F. armeniacum* strain were also detected. A subset of 61 FFSC strains were analyzed for *in vitro* production of fumonisins (FBs), beauvericin (BEA), and enniatins (ENNs). *Fusarium proliferatum* was the most toxigenic species, since 36 out of 44 strains produced FBs. In particular, FB1 was the most produced mycotoxin with values ranging from 4 to 6072 µg g^{-1}. All FB1 producer strains synthetized also FB2 (up to 1100 µg g^{-1}) and FB3 (up to 2127 µg g^{-1}). Only 14 *F. proliferatum* strains produced BEA (up to 71.6 µg g^{-1}) and low levels of ENNB and ENNB1. *Fusarium sacchari* synthetized only very low level of BEA and B ENNs; *Fusarium* sp. strains produced only B ENNs. This study provides new insights on the *Fusarium* species and their mycotoxin profile occurring on sugarcane in Iran.

THE DISEASE CYCLE OF FUSARIUM HEAD BLIGHT OF WHEAT IN DIFFERENT ECOLOGICAL REGIONS IN CHINA

ZHANG H.

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing, China

zhanghao@caas.cn

In the last decades, Fusarium head blight (FHB) outbreaks have occurred much more frequently in China. The knowledge of disease cycle of Fusarium pathogen is important for developing effective management strategies. The change of tillage system is suggested to contribute to more severe epidemics as it may increase the initial inoculum. In this study, we investigate the species composition and potential mycotoxin of saprophytic *Fusarium* on different plant debris in the main wheat producing areas. Rice, maize and soybean was the predominant overwinter vector in rice-wheat, maize-wheat and soybean-wheat rotation system respectively. Along with the increasing application of herbicide, more and more weed debris were left in the field and easily infected by *Fusarium*, which was confirmed as the secondary inoculum of FHB. The different crops rotate with wheat also showed specific-specific selective pressure for the pathogen. In addition, the relation between Fusarium disease of rotation crops and FHB on wheat was surveyed, which may be important for survival of FHB pathogen after wheat season. The composition of pathogen of maize ear rot, maize stalk rot, Fusarium crown rot of wheat and soybean root rot varied, but FHB causing species were also the important parts of these diseases. This indicated rotation systems significantly related with FHB at different levels. Overall, we identified the inoculum and related diseases of FHB in different cropping systems, this will be helpful for extending the period of FHB management.

This research was financially supported by National Key Research and Development Program (2016YFE0112900) and MycoKey-Project (EU-H2020-678781).

to Index

Session 2

Challenges in Mycotoxin Analysis in food and feed

HOW AND WHY SHALL WE DETERMINE >500 BIOTOXINS IN FOOD AND FEED?

KRSKA R. [1,2] , SULYOK M.[1]

[1]*University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria;* [2]*Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom*

rudolf.krska@boku.ac.at

Monitoring of food contaminants and residues has undergone a significant improvement in recent years and is now performed in an intensive manner [1]. Achievements in the area of chromatography-mass spectrometry coupling techniques enabled the development of quantitative multi-target approaches covering several hundred analytes. This paper provides an overview of relevant multi -analyte concepts based on LC-MS/MS instruments. Merits and shortcomings will be critically discussed based on current performance characteristics of the EU legislation system. In addition, the discussion of a recently developed approach covering >500 secondary microbial metabolites including relevant biotoxins is presented as a case study to illustrate the current developments in this area and their employment in food and feed analysis. Analytical performance parameters have been determined for seven food matrices using seven individual samples per matrix for spiking. Apparent recoveries ranged from 70 to 120% for 53–83% of all investigated analytes (depending on the matrix) [2]. This number increased to 84–94% if the recovery of extraction was considered. Based on these findings, this paper also discusses the applicability and practicability of current guidelines for multi-analyte method validation. A major conclusion of our studies is clearly that more emphasis should be put on the investigation of relative matrix effects in the validation procedure.

NON-DESTRUCTIVE DETECTION OF AFLATOXINS B IN GRAINED ALMONDS USING FLUORESCENCE SPECTROSCOPY AND MACHINE LEARNING ALGORITHMS

BERTANI F.R.[1], GAMBACORTA L.[2], MENCATTINI A.[3], DE NINNO A.[1], SOLFRIZZO M.[2], MARTINELLI E.[3], GERARDINO A.[1*]

[1]Consiglio Nazionale delle Ricerche, Istituto di Fotonicae Nanotecnologie, via Cineto Romano 42, 00156, Roma, Italy; [2]Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari, via Amendola, 122/O, 70126, Bari, Italy; [3]Departmentof Electronic Engineering, University of Rome Tor Vergata, via del Politecnico 1, 00133, Roma, Italy

annamaria.gerardino@cnr.it

Optical approaches are recently being developed to assess presence of aflatoxins contamination ina cost and time effective way, maintaining acceptable accuracy and reproducibility. In this work we present the results obtained with a simple portable device for non-destructive detection of aflatoxins in almonds developed during the PhasmaFOOD H2020 project. The presented approach is based on the analysis of fluorescence spectra of slurried almonds under 375 nm wavelength excitation. Experiments were conducted with almonds contaminated in the range of 2.7–320.2 ng/g total aflatoxins B (AFB1 + AFB2) as determined by High Performance Liquid Chromatography with Fluorescence Detection (HPLC/FLD). After applying pre-processing steps, spectral analysis was carried out using a binary classification model based on Support Vector Machine (SVM) algorithm. A majority vote procedure was then performed on the classification results. In this way we could achieve, as best result, a classification accuracy of 94% (and false negative rate 5%) with a thresholdset at 6.4 ng/g. More recently we are exploring a new strategy based on the analysis of images of the contaminated almonds samples under UV illumination, as to say the fluorescence imaging. The preliminary results will be also described. These results illustrate the feasibility of such approach in the great challenge of aflatoxin detection for food and feed safety.

This research was financially supported by the Project H2020 PhasmaFood contract n. 732541

AFLATOXIN ANALYSIS IN MILK WITH WORLD'S FIRST AOAC PERFORMANCE TESTED METHODS[SM]

ROSAR G.
Eurofins Tecna, part of Eurofins Technologies, c/o Area Science Park, Padriciano 99, Trieste, Italy
GiuliaRosar@eurofins.com

Aflatoxin M1 (AFM1) is the major metabolite of Aflatoxin B1 (AFB1) and can be found in the milk of animals fed with feed containing AFB1. The frequency of occurrence of AFM1 in milk has led to the development of specific quantitative methods of analysis to mitigate the risk of adversely affecting human health. I'screen AFLA M1 milk is a quantitative ELISA test kits for the analysis of AFM1 in raw and powdered milk that has been commercialized since the Italian outbreak in 2003. In 2020, the kit was granted AOAC Research Institute Performance Tested Methods[SM] status (AOAC Cert. No. 072002) for use with raw bovine whole milk, skim milk and powdered milk. To get the approval, Assay performance was evaluated studying lot-to-lot consistency, assay stability, robustness, and possible interferences of related molecules. Raw bovine milk samples spiked at 0, 5.0, 20, 50, 100, and 200 ng/L of AFM1 and powdered milk reference materials and spiked samples at 100 and 200 ng/L were tested to determine recovery, repeatability, and bias. LOD and LOQ were also determined for both matrices. The matrix study was confirmed by QLAB, and independent laboratory in US, that used for raw milk verification local, fresh materials. As a result, the kit showed high selectivity for AFM1, robust and stable performances, comparable performances among laboratories, high sensitivity. Recoveries for spiked raw and powdered milk were 97-122%, with RSDr < 10%, and 106-111% for reference materials, with RSDr < 5%.

to Index

ANALYTICAL CHALLENGES ASSOCIATED WITH DETECTION OF EMERGING, MASKED, AND MODIFIED MYCOTOXINS

MARAGOS C.
Mycotoxin Prevention & Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, IL, 61604, USA
chris.maragos@usda.gov

All mycotoxins were, at one time, "emerging" toxins. Those toxins that were identified many years ago, such as the aflatoxins, deoxynivalenol, ochratoxin A, zearalenone, and the fumonisins, have each been associated with diseases in humans or domestic animals. Over time the tools of both analytical chemistry and toxicology have improved significantly, culminating in modern tools that permit the better discernment and quantification of the additive and synergistic effects resulting from exposures to multiple toxins and environmental influences. This has enabled the shift from using a reactive approach to a proactive approach for limiting mycotoxin exposures. This shift has also led to many challenges for analtyical chemists. Some of the challenges are technical in nature and would be recognizable to the analytical chemists of the past: finding the best way to extract toxins of very different polarities, reducing matrix effects, achieving adequate sensitivity, and performing validations. Investigations into how the well known mycotoxins interact with plants and animals has led to the discovery of modified, masked, and matrix-associated forms. Identifying all of the myriad forms is a significant challenge. In addition to direct technical challenges there are also challenges that are non-technical in nature. Foremost among these is establishing which of the many potentially hazardous materials need to be monitored, which in turn is based upon toxicological relevance and the presence or absence of regulatory guidelines. Establishing relevance depends upon the availability of accurate measurement tools. That is, the development of analytical tools must be undertaken before the extent of a potential hazard can be fully characterized.

This research was financially supported by the USDA-Agricultural Research Service Project 5010-42000-049-00D.

ALTERNARIA TOXINS AND ERGOT ALKALOIDS IN FOOD BY LC-MS/MS: RESULTS OF THREE YEARS MONITORING IN UMBRIA AND MARCHE REGIONS (CENTRAL ITALY)

SDOGATI S. , FIORONI L., BIBI R. *, CAPORALI A.,
PAOLONI A., VERDINI E., PECORELLI I.

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via Salvemini 1, 06126-Perugia, Italy.

ir.bibi@izsum.it

Alternaria toxins are a group of more than 70 phycotoxins produced by *Alternaria* species molds. Some of them such as Alternariol, Alternariol monomethyl ether, Tenuazonic acid, Tentoxins and Altenuene, due to their adverse effect on humans, are of concern for public health. Ergot Alkaloidsare produced by fungi of the genus *Claviceps* and 12 of them (Ergocristine, Ergotamine, Ergocryptine, Ergometrine, Ergocornine, Ergosine and their related -inines) should be monitored, according to EU legislation, because their effects are harmful to humans and animals[1]. Alternaria Toxins and Ergot Alkaloids are not yet included in Regulation (EU) 1881/2006 setting maximum levels for certaincontaminants in foodstuff. The development and validation[2] of two LC-MS/MS analytical method was supported by Italian Ministry of Health (Research Project RC008/2016 IZSUM) and used in the participation to two interlaboratory studies under CEN Mandate M/520. During the years 2017/2019 about 80 samples of tomato products, cereals and sunflower seeds, were collected in two Italian Regions (Umbria and Marche) and analyzed to determine Alternaria toxins andErgot alkaloids and results were sent to EFSA for risk assessment. The analyses resulted in high contaminations of food products by Alternaria toxins (80% overall)[3] and risks to the health of consumers cannot be excluded, while Ergot Alkaloids were detected in a significantly lower percentage of samples compared to Alternaria toxins. Moreover, the manufacturers should be made aware of the necessity to screen their products more thoroughly for these toxins and take the appropriate measures to reduce their contents.

This research was financially supported by Italian Ministry of Health, Department of Veterinary Public Health and FoodSafety. Rome, Italy. RC008/2016 IZSUM.

ELISA TEST KITS PERFORMANCE MONITORING THROUGH REGULAR PROFICIENCY TEST PARTICIPATION AND CONTROL MATERIAL IMPLEMENTATION

BRAVIN F.*, BASSANI V., RUNTI G., ROSAR G.

Eurofins Tecna, part of Eurofins Technologies, , c/o Area Science Park, Padriciano 99, Trieste, Italy.

FrancescaBravin@eurofins.com

Eurofins Tecna regularly attends every year to a series of Proficiency Tests (PT) with self-developed ELISA systems. EN ISO/IEC 17043:2010 accredited circuits are preferred and, among them, those providing naturally incurred materials and a clear description of how the participants' consensus is used and elaborated to define the material assigned value.

Experiments are run by R&D scientists, QA operators and the commercial technical assistance team, thus assuring to involve multiple operators with different experience.

Samples are stored and treated according to the PT provider instructions and then extracted following the procedure included in the kit insert. The unknown material is extracted twice if feasible; each extract is run in triplicate in two separate runs. The mean result is submitted according to the PT provider requirements.

In the present work, Eurofins Tecna reports about 32 traceable results obtained for ELISA kits for mycotoxins analysis in vegetal samples, plus 110 traceable results obtained with ELISA kits for aflatoxin M1 detection in milk and dairy products. 100% results were satisfactory, with given Z-score within -2 and + 2, while 84% had Z-score included in the range -1 and +1. Left over materials with assigned value given by means of a PT are useful tools to monitor the quality of assays over time, qualify the new personnel and compare the results among different sites.

USE OF ELECTRONIC NOSE TO DETECT AFLATOXIN AND FUMONISIN IN MAIZE

M. CAMARDO LEGGIERI[1], M. MAZZONI[1], S. FODIL[1] M. MOSCHINI[2], A. PRANDINI[2], T. BERTUZZI[2] AND P. BATTILANI[1]

[1]Department of Sustainable Crop Production, [2]Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, via E. Parmense 84, 29122 Piacenza, Italy.

paola.battilani@unicatt.it

Mycotoxin poses a significant threat to the safety of food and feed products, and maize represents one of the most susceptible crops. To manage this issue, fast, reliable and cheap testing methods are needed for the most important regulated mycotoxin. This study aimed at evaluating the potential use of the electronic nose for the rapid identification of maize samples contaminated by mycotoxins above the legal limits. A total of 316 maize samples were collect from commercial field in Northern Italy from 2014 to 2018 and analysed both for aflatoxin and fumonisin contamination with a conventional method (HPLC-MS) and using a portable e-nose "AIR PEN 3" (Airsense Analytics GmbH, Schwerin, Germany) equipped with 10 metal oxide sensors for different categories of volatile substances. Neural network (NN), logistic regression (LR) and discriminant analysis (DA) were used to investigate whether the e-nose was able to separate samples contaminated above or below the legal limit of AFB1 and FBs (EC Reg. 1881/2006). The statistical methods adopted were externally validated using ≈ 20% of the total dataset. All the methodologies used showed high accuracy (≥70%) in distinguish maize grain contaminated above or below the legal limit. Above all, NN performed better than the other methods, with 78 and 77% accuracy respectively for AFB1 and FBs. This is the first time that three different approaches were adopted to check e-nose performances and pros and cons discussed. The obtained results suggest that the e-nose can be adopted as rapid method to discriminate between healthy maize lots and those contaminated with mycotoxins.

SENSOR SURFACE FUNCTIONALISATION *VIA* LASER INDUCED FORWARD TRANSFER (LIFT): ELECTROCHEMICAL PESTICIDE DETECTION IN OLIVE OIL AND ITS POTENTIAL USE IN OTHER APPLICATIONS

CHANDRINOU C.[1], MANDALA D.[1], TSEKENIS G.[2], SOULIS D.[2], ZERGIOTI I.[1*].

[1]School of Mathematical and Physical Sciences, Physics Department, National Technical University of Athens, Heroon Polytehneiou 9, 157 80, Athens, Greece; [2]Biomedical Research Foundation Academy of Athens, 4 Soranou Ephessiou Street, 115 27, Athens, Greece.

zergioti@central.ntua.gr

Food monitoring, at any stage of the production chain, is becoming increasingly important, as health issues relating to the contamination of food is becoming more prominent. This creates a need for detection methods that not only produce fast and reliable results but also have the potential to be implemented in portable devices that can be deployed at the Point-of-Need. Electrochemical sensors are increasingly used for the detection of various analytes (pesticides, mycotoxins, plasticizers etc.) in food, for rapid and reliable detection without the need for expensive instrumentation and skilled personnel. Herein, we describe the use of Laser Induced Forward Transfer (LIFT) for the highly-precise functionalization of SPEs for the fabrication of electrochemical sensors for the detection of pesticides in olive oil. The sensor was developed *via* the LIFT technique that possesses significant advantages in comparison to the conventional drop-casting method for sensor fabrication. With LIFT bio-printing at 355 nm, delivery of the biomolecules and/or the signal enhancement matrix can be achieved in one step, with high special resolution, thus allowing extremely small electrode surfaces to be printed, ultimately reducing consumables waste. In addition, the high impact pressure of the transferred droplets improves the electrochemical communication with the screen-printed electrodes and improves reproducibility. Employment of the optimized sensor fabrication allowed the sensitive detection of both carbamate and organophosphate pesticides in pretreated olive oil samples at values below the legislation limit of 10 ppb. A plethora of other contaminants in different food matrices can be detected by changing the biorecognition element.

The research activities that led to these results were co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: Τ1ΕΔΚ-04360).

RAPID SCREENING OF OCHRATOXIN A IN WHEAT AND DEOXYNIVALENOL IN WHEAT BRAN BY INFRARED SPECTROSCOPY

DE GIROLAMO A.[1], CERVELLIERI. S.[1], CORTESE M.[1], VON HOLST C.[2], PASCALE M.[1], PORRICELLI A.C.R.[1], LIPPOLIS V.[1] AND LOGRIECO A.F.[1].
[1]*Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Bari, Italy.* [2]*European Commission, DG Joint Research Centre, Geel, Belgium.*
annalisa.degirolamo@ispa.cnr.it

Ochratoxin A (OTA) and deoxynivalenol (DON) represent the most prevalent mycotoxins in terms of toxicity and frequency of contamination of wheat and derived products. In order to preserve the consumer's exposure to mycotoxins, the European Commission has defined maximum admissible levels of mycotoxins in cereals and derived products (EC Regulations No. 1881/2006 and 1126/2007). For this reason, it is necessary to have reliable screening methods to assess the compliance of the food with the legislation in force and reduce the number of samples to be submitted to confirmatory analysis.

In the present work, the potential of infrared spectroscopy for the screening of wheat samples naturally contaminated with OTA and wheat bran samples naturally contaminated with DON was investigated. Samples were analyzed by both Fourier transform near- and mid-infrared spectroscopy (FT-NIR, FT-MIR), in combination with multivariate statistical analysis, to classify contaminated wheat and wheat bran samples based on their mycotoxin content and prediction results were compared. For OTA, the use of a cut-off limit set at 2 µg/kg provided prediction rates between 94-96% for both FT-NIR and FT-MIR ranges. For DON, the use of a cut-off limit set at 400 µg/kg DON provided prediction rates between 86-91% with the FT-NIR performing better than the FT-MIR range.

The high prediction rates and the absence or low percentages of false compliant samples indicated that the infrared spectroscopy might be a promising, inexpensive and easy-to-use screening tool to rapidly discriminate wheat samples for OTA content and wheat bran samples for DON content and verify the compliance with the EU regulatory levels.

DEVELOPMENT AND VALIDATION OF TOF/Q-TOF MS/MS, HPLC METHOD REVEALED NOVEL MYCOTOXIN METABOLITES

FETO N.A.[3], MITEMA A.[1, 2, 3], RAFUDEEN M.S.[1]

[1]Department of Molecular and Cell Biology, Plant Stress Laboratory 204/207, MCB Building, Upper Campus, University of Cape Town, Private bag X3, 7701, Rondebosch, Cape Town, South Africa; [2]School of Biological Sciences, University of Nairobi, P.O. Box 30596-0 0100, Nairobi, Kenya; [3]Department of Biotechnology, OMICS Research Laboratory, Vaal University of Technology, South Africa.

alfmite@yahoo.com; suhail.rafudeen@uct.ac.za; naserf@vut.ac.za

Mycotoxins are secondary metabolites produced by fungi, *Aspergillus* species. They are carcinogenic, hepatotoxic and cause birth defects in humans and animals. We developed and optimised bio-analytical tools for detection of metabolites, aflatoxins and to evaluate effectiveness of the methods in co-infected maize tissues. Acetonitrile/methanol/formic acid (9:1:0.2 v/v) gave better mycotoxins separation. Derivatisation by TFA exhibited gradient suitable for positive electrospray ionisation. HPLC chromatograms revealed retention times for aflatoxins AFG1 (11.39-11.68); AFB1 (12.72-12.84); AFG2 (17.71-17.80) and AFB2 (18.73-18.91). The non-toxin producing strain (isolate KSM012) demonstrated no HPLC peaks and no blue fluorescence on TLC plate confirming the non-toxicity. Isolate KSM015 produced AFB1 and AFB2 in addition to AFG1 and AFG2 respectively an indication of possible S_{BG} morphotype. The LOD and LOQ ranged from 0.01–6.8 µg/ml and 0.02–35.81 µg/ml respectively. Though TLC detected the presence or absence of aflatoxins, it was not able to measure the LOD and LOQ. The best chromatograms with lowest noise was obtained at 100 % acetonitrile and ultra-pure water spiked with 0.1 % FA at a flow rate of 0.3 ml/min. Application of +ve and -ve ion modes with electrospray ionisation exhibited better fragmentation for mycotoxins. LC-MS/MS detected 17 aflatoxins/derivatives by targeted and formula mass. The detection limits, sensitivity and linearity showed the method developed was acceptable for mycotoxin determination in comparisons to the guidelines of European Commision 657/EC 2002.

IS THERE A SIMPLE, VERSATILE AND RELIABLE AUTOMATION SOLUTION FOR THE QUANTIFICATION OF TOXINS ON FEED AND FOOD MATRIXES? AN EASIER WAY TO GET RELIABLE RESULTS

GALIAZZO G.
Futurlab SrlVia Pierobon, 63 – 35010 Limena (PD) - Italy
giovanni.galiazzo@futurlab.it

Food safety and salubrity is currently a topic of maximum significance, which conveys the consumers' choices and responses in their everyday life. Determining the presence of toxins in food and feed matrixes is thus a fundamental objective for guaranteeing the final consumer's health and for preventing risks. In order to ensure safety, the request for analytic controls in laboratory practice is increasingly growing.
Toxins can be quantified through various analytical techniques, such as mass spectrophotometry or immunoassay, as well as through other less accurate devices, like lateral flow immunochromatographic assays. The main disadvantage of all of the techniques above mentioned is the employment of dedicated Operators that have to be always focused on the test running, an approach that often keeps them from doing other relevant activities in the Lab.
Even though the amount of analytic controls has been growing in the last years, the automation of these procedures has not followed this direction, as many steps that should be standardized, like the preanalytical phase, are still performed manually, thus being subjected to mistakes, slowdown and economic loss.
Nowadays, the market offers to the majority of Laboratories versatile and accessible solutions that help Practitioners in this valuable diagnostic task.
These Automade systems allow to reduce error risks due to manual procedures during the tests running, without negative effects on the quality of the analytical data obtained.

RAPID AND SENSITIVE LC–MS/MS ANALYSIS OF LESGISLATED MYCOTOXINS IN FEED AND FOOD SAMPLES

RODRIGO D, CANTALAPIEDRA A., GALLEGO L.*
Chromatografy Departament. Analiza Calidad Madrid S. L. Analiza Calidad Group
luisg@analizacalidad.com

Different mycotoxins have been identificated, but the most commonly observed are aflatoxins, ochratoxin A, trichothecenes and zearalanone. Mycotoxins appears in the food chain in the storage of cereals and raw material. However, climate change will affect the development of cereal crops and the occurrence of mycotoxins in these crops. Several studies has shown increase of deoxynivalenol in wheat, aflatoxin B1 in maize or ochratoxin A in coffee. A multimycotoxin LC-MS/MS method was developed and validated according to ISO 17025:2017 for the simultaneous detection and quantification of 11 mycotoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, deoxynivalenol, T-2 and HT-2 toxin, fumonisin B1, fumonisin B2 and zearalenone) in cereal, raw material and feed. Mycotoxins were extracted in a single step with a mixture of acetonitrile:water:acetic acid (79:19:1 v/v/v) and this extract was purificated with clean-up columns. Mycotoxins were separated with reversed-phase column and detected using an electrospray ionization interface (ESI) and tandem MS. The method limits of quantification (LOQ) varied from 1.0 to 100 µg/kg. The limit of quantification for the aflatoxins and ochratoxin A was 1.0 µg/kg, zearalenone 30 µg/kg, T-2 and HT-2 toxin 10 µg/kg, fumonisins 100 µg/kg and for deoxynivalenol 50 µg/kg. Good precision and linearity were observed for most of mycotoxins. The analysis of seven different matrices spiked (maize, wheat, bread, nuts, almonds, feed and pet food) provided a good basis for the evaluation of the toxin exposure in storage of these products. In a future, this procedure may be extended to other mycotoxins and products.

to Index

SURVEY OF AFLATOXINS IN COCOA POWDER SAMPLES

HERRERA M. *, SALAS G.[1], LORÁN S., CARRAMIÑANA J.J., JUAN T.[1], HERRERA T., YAGÜE C. ARIÑO A.

Instituto Agroalimentario de Aragón-IA2, Veterinary Faculty, University of Zaragoza-CITA, 50013 Zaragoza, Spain.

herremar@unizar.es

Aflatoxins represent a serious risk to food safety, contaminating crops in the field and during storage and affecting a wide variety of raw materials and processed foods. Furthermore, the incidence in the EU food supply chain has increased in recent years by factors related to the climate change. Foodstuffs contaminated with aflatoxins are a major threat to food safety, potentially affecting the most vulnerable population groups, such as children. Therefore, it is important to investigate the occurrence of aflatoxins in cocoa, which is frequently consumed by this high-risk population group. In addition, there is currently a certain legal gap in EU legislation since the content of aflatoxins B1, B2, G1 and G2, which are carcinogenic to humans, is not regulated for cocoa and derived products. The aim of this study was to evaluate the contamination by aflatoxins B1, B2, G1 and G2 in 63 commercial samples of branded cocoa powder (12 organic and 51 conventional). Mycotoxins were extracted with methanol: water (80:20) followed by cleanup using immunoaffinity columns. Finally, the determination was made by HPLC coupled to photochemical (PHRED) and fluorescence (FLD) detectors, with a limit of detection of 0.02 µg/kg for each of the aflatoxins. Thirty-two out of 63 samples were positive to total aflatoxins, with levels ranging from 0.02 µg/kg to 3.39 µg/kg. The incidence of the different aflatoxins was B1 (23 samples), G1 (14 samples) and B2 (6 samples); no aflatoxin G2 was detected. Aflatoxins B1, B2 and G1 were detected simultaneously in three samples, B1 and G1 in six samples, while B1 and B2 coexisted in five samples. The incidence of total aflatoxins was very similar in organic (50%) and conventional (51%) cocoa samples.

This research was financially supported by Government of Aragón and FEDER 2014-2020 (Group A06_17R).

SIMULTANEOUS ANALYSIS METHOD OF MYCOTOXIN USING REAGENT PRE-TREATMENT INJECTION METHOD ON LC-MSMS

JO H.W.[1], HWANG K.W.[2], KWAN MOON J.[2].
[1]*Hankyong National University Industry Academic Cooperation Foundation, 327, Joongang-ro, Anseong-si, 17579, Republic of Korea;* [2]*Departament of Plant Life Environmental Science, Hankyong National University, 327, Joonang-ro, Anseong-si, 17579, Republic of Korea.*
hyeongwook.jo@hknu.ac.kr

Simultaneous analysis method of mycotoxin using reagent pre-treatment injection method on LC-MSMS from animal feed for monitoring was developed with QuEChERS preparation. After sample weighing (5g) in a 50 mL conical tube, 10% formic acid in distilled water (10 mL) and acetonitrile (10 mL) was added. After it was strongly shaken for 30 min, QuEChERS extraction salt (4 g $MgSO_4$, 1 g NaCl) were added to the 50 mL conical tube. The mixture was strongly shaken for 1 min and was centrifuged at 3,000 *G* for 10 min. The acetonitrile layers (1 mL) were purified by dSPE (50 mg C_{18}, 50 mg PSA) and centrifuged at 10,000 *G* for 5 min. The purified extract was filtered with a membrane filters (pore size: 0.2 um) before analysis. For quantitative analysis, dilution, using internal standards (mycotoxin isotope), and matrix matched calibration curves should be used to reduced matrix effect (ME). Dilution has a disadvantage of increasing the method limit of quantitative and can`t matrix matched calibration curves every time because of the variety of matrix. Also, internal standards can`t add to all samples due to it was expensive. Therefore, this study applied reagent pre-treatment injection method (the reagent reaction method using autosampler) like the amino acid analysis using HPLC.

This work was supported by Korea Institute of Planning and Forestry (IPET) through Agro and Livestock Products Safety · Flow Management Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (318071-3)

IMMUNOASSAY OF FUSARIUM FOR ROUTINE CONTROL OF STORED GRAIN

LEBEDIN Y.1, GAGKAEVA T.2, ORINA A.2, GAVRILOVA O.2
[1]*XEMA Company Limited, 105264, Moscow, Russia;* [2]*All-Russian Institute of Plant Protection (VIZR), 196608, St.-Petersburg, Pushkin, Russia.*
lebedin@xema-medica.com

Routine control of Fusarium infection of stored grain is based on visual examination, and depends on operator skills. Direct quantification of fungal DNA by PCR and determination of mycotoxins by chromatography are time-consuming methods that require specific conditions and expensive equipment. Immunoassays, especially in rapid test format, may represent a convenient screening tool for detection of fungal contamination and potential risk of mycotoxins in cereals. The aim of study was to evaluate immunoassays for detection of F. graminearum in simulated grain samples with a strict proportion of infected and non-infected grains. Initially, the absence of Fusarium fungi in wheat grain samples was proven by mycological method and qPCR. Then artificially infected by F. graminearum grains were added to these samples in the percent ratios: 0 (control); 0.1; 0.5; 1.0; 3.0; 5.0. Enzyme immunoassay in competition format and rapid test (respectively Fusarium antigen ELISA, cat# K827 and XEMATest Fusarium, cat# X827; both from XEMA, Russia) were used for evaluation of F. graminearum biomass in the grain samples. The qPCR with TaqMan probe was used as confirmation method of fungal biomass detection. DON amount was measured by ELISA (cat# K925; XEMA, Russia). The high positive correlations ($p<0.05$) were revealed between data obtained by different methods: $r=+0.80$-0.92 in the case of Fusarium antigen ELISA and $r=+0.59$-0.77 in the case of rapid test. Simple extraction and fast procedure of rapid test allowed detection of the 0.1% admixture infected grains. Fusarium immunoassays can be recommended for efficient routine control of grain.

FLUORESCENCE POLARIZATION IMMUNOASSAYS FOR THE DETERMINATION OF TRICHOTHECENES AND THEIR MODIFIED FORMS IN WHEAT

LIPPOLIS V.1, PORRICELLI A.C.R.1, MANCINI E. 1, LATTANZIO V.M.T.1, CIASCA B.1, DE GIROLAMO A.1, DE SAEGER S.2, MARAGOS C.M.3, MCCORMICK S.3, LI P.4, PASCALE M.1, LOGRIECO A.F.1.

[1]Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Bari, Italy; [2]Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium; [3]Mycotoxin Prevention and Applied Microbiology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, USA; [4]Key Lab for Mycotoxins Detection, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan 430062, China.

vincenzo.lippolis@ispa.cnr.it

Deoxynivalenol (DON), T-2 toxin (T-2) and HT-2 toxin (HT-2), due to their incidence and toxicity, are the trichothecenes of major concern for cereals and cereal-based products. Moreover, natural occurrence of modified forms of DON, such as 3-acetyl-deoxynivalenol (3Ac -DON), 15-acetyl-deoxynivalenol (15Ac-DON), and deoxynivalenol-3-glucoside (DON3G), and of T-2 and HT-2, such as T-2 glucoside (T-2G) and HT-2 glucoside (HT-2G), have been reported by several authors. The development of analytical methods for the simultaneous detection of these mycotoxins and their modified forms, also expressed as the sum, is highly requested since it could meet future requirements of European or international regulations. For this reason, two fluorescence polarization immunoassays (FPIAs) have been developed and in-house validated for the rapid (<15 min) and simultaneous determination, expressed as sum, of: (i) DON, 3Ac-DON, 15Ac-DON and DON3G in wheat and (ii) T-2, HT-2, T-2G and HT-2G in wheat. The developed FPIAs showed good analytical performances in terms of recovery (89-112%) and precision (≤13%) fulfilling the EU criteria for acceptability of an analytical method for the determination of relevant native forms. The assays have been also validated according to the harmonized guidelines for the validation of screening methods (Regulation EU, No 519/2014). The satisfactory analytical performances, in terms of precision (repeatability ≤9%, within-laboratory reproducibility ≤13%), cut-off levels and rate of false positive results (<0.1%), confirmed the applicability of the proposed FPIAs as methods for the high-throughput screening of these trichothecenes and their modified forms in wheat. Moreover, it is highlighted that the developed FPIAs are low-cost, portable, can be automated and do not require a high level of technical skills.

This work has been supported by the MYCOKEY project "Integrated and innovative key actions for mycotoxin management in the food and feed chain" (H2020 - Grant Agreement No 678781).

to Index

A SYSTEMATIC REVIEW OF THE TRANSFORMATION AND DETECTION METHODS OF MODIFIED MYCOTOXINS

LU Q., QIN J.A., FU Y.W., LUO J.Y.*, YANG M.H.

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences &Peking Union Medical College, Beijing 100193, China;

luqian0313@foxmail.com

Mycotoxin contamination is one of the major hazards towards global food, feedstuff and traditional Chinese medicines (TCMs) safety, and has the potential harm to animals and humans. In addition to being present in contaminated foods and TCMs, mycotoxins can also exist in a modified form, called modified mycotoxins. They are the phase II metabolites of mycotoxins originally formed by the defense mechanisms of plant, mainly included conjugates with glucoside and sulfate and other acetylation products. Modified mycotoxins are usually stable and widely distributed, the occurrence of them has been reported in many countries, e.g. America, China and Japan. According the toxicological data, few of these modified mycotoxins can be even more toxic, for their higher affinity and bioavailability. Besides, they can be further transformed into the parent mycotoxins, with clearly increasing total exposures and pose additional threat to animals and human. Compared with the parent mycotoxins, the modified forms undergo significant changes in polarity, solubility, and chemical properties, making them difficult to be detected using routine analysis methods. Therefore, the task of accurately quantifying modified mycotoxins is rather urgent. This review seeks to provide information about the common detection methods and transformation of modified mycotoxins. This work will provide a basis for the detection of modified mycotoxins in the future, and also play an important role in the early diagnosis, warning, safety evaluation and control of modified mycotoxins, with the goal of better protecting TCMs safety and human health.

This research was financially supported by the Project 2017-I2M-1-013 (Institute of Medicinal Plant Development, Beijing, China)

INSIGHTS ON GLOBAL OCCURRENCE OF FREQUENTLY FOUND EMERGING OR MASKED MYCOTOXINS IN 2019 AS DETECTED BY SPECTRUM TOP® 50

MUELLER A.[1]*, TASCHL I.[1], JENKINS T.[1], HASLER K.[2], HOFSTETTER U.[1].
[1]*BIOMIN Holding GmbH, Getzersdorf, Austria.* [2]*Romer Labs Diagnostic GmbH, Technopark 5, 3430 Tulln, Austria.*
anneliese.mueller@biomin.net

In 2018, BIOMIN and Romer Labs introduced an innovative Multi-Mycotoxin Analysis, Spectrum Top® 50, allowing the simultaneous detection of over 50 different major mycotoxins and the less-studied "emerging mycotoxins". With 2209 samples from the Americas, Europe, Africa and Asia analyzed in 2019, insights of occurrence of each metabolite were gained. We describe the eight most common metabolites detected in the important crops corn, corn silage and wheat. In 92% of corn samples (n=542), beauvericin was found (average of positives 32 ppb, maximum 744 ppb). Also prevalent were moniliformin (40%, 246 ppb, max. 1855 ppb), the masked mycotoxin deoxynivalenol-3-glucoside (48%, 131 ppb, max. 2196 ppb), as well as fumonisins (FUM B1, B2 and B3), deoxynivalenol (DON) and zearalenone (ZEN). In corn silage (n=183 from Europe, Asia, Africa), enniatins were highly prevalent including enniatin B (81%, average 105 ppb, max. 2499 ppb), B1 (69%, 10 ppb, max. 82 ppb), A1 (62%, 8 ppb, max. 239 ppb) and A (40%, 3 ppb, max. 27 ppb). FUM (B1 and B2), ZEN and DON were also prevalent in corn silage. In Asian corn nivalenol was abundant (55%, average 178 ppb) as was in Asian corn silage sterigmatocystin (50%, average 161 ppb). Wheat (Europe, Africa, Asia, n=158) was frequently contaminated with enniatins (B1, A1 and A), DON-3-glucoside, beauvericin and moniliformin, as well as DON and FUM B2. Wheat in Asia and South Africa showed relatively high prevalence of ergot alkaloids (>35%). High abundance and co-occurrence underlines the need to monitor more than the six major mycotoxin groups.

CLOSING THE GAP: SMARTPHONE BASED ANALYTICAL TOOLS FOR MYCOTOXIN TESTING

NIEMEIJER R.
R-Biopharm AG, An der neuen Bergstrasse 17, Darmstadt
r.niemeijer@r-biopharm.de

Mycotoxins have a major economic impact. According to the World Health Organization (WHO) still more than 25% of the world's crops are contaminated with mycotoxins. Mycotoxins cause serious health issues to a significant part of the human population. Maybe not in the western world, where mycotoxin management has been very effective, but mycotoxins in staple foods are a serious health risk for consumers in Asia, Africa and Latin America. Mycotoxins still cause significant losses of efficiency in livestock farming and maybe a health risk for pets. Consumers are getting more concerned about the quality of food and regulations are increasingly enforced globally. Mycotoxins contaminations of crops are unavoidable but mycotoxins can be managed. Good agricultural and good manufacturing practices will help. Monitoring mycotoxin contaminations by testing is necessary to verify the products will meet international regulations and guidelines. Yet, instead of testing large numbers of end-products, a more pro-active approach would have many benefits.

The use of mobile devices in mycotoxin analysis and sharing the analytical data in the cloud has opened entirely new ways of mycotoxin data use. Analytical data about the quality of commodities can be available from all locations in real-time. The analytical data may also be used in combination with other agricultural and environmental data, like weather conditions enabling to create more precise predictive models.

During the entire process from field to food or feed critical steps can be identified to monitor mycotoxins. For this approach a mobile, easy to use tool to make quick, on-site decisions is essential. Yet equally important is that these decisions are made in a reliable way so that the quality of the method is assured.

CERTIFIED REFERENCE MATERIALS AND QUALITY CONTROL MATERIALS: VALUABLE TOOLS FOR LABORATORY MANAGEMENT

MAUNE C.[1*], MALONE R.[1], RODGERS J.[1], BRUNKHORST J.[1], NIEMEIJER R.[2*]
[1]*Trilogy Analytical Laboratory, 870 Vossbrink Dr., Washington, MO 63090, USA, E-mail:.* [2]*R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany,*
carrie@trilogylab.com; r.niemeijer@r-biopharm.de

Quality management for ISO 17025 accredited facilities is an inherent part of daily operations. Utilizing Certified Reference Materials (CRM) that have been produced by an ISO 17034 accredited reference material producer (RMP) in tandem with Quality Control Materials (QCM) as a daily check system are cornerstones of a well-rounded quality system.
Both CRMs and QCMs are crucial tools for calibrations, method validations, analyst training, defining acceptance criteria, blind testing, troubleshooting, and proficiency testing. Exploring new uses for these materials has the potential to further enhance quality systems. In the following this poster will detail a few Trilogy recommendations for incorporation of CRMs and QCM's into a quality system to create a total quality framework that will ensure and validate confidence in your analysts, results and methods

THE EFFECT OF GRIND AND EXTRACTION SIZE ON DEOXYNIVALENOL RESULT VARIABILITY

MAUNE C.[1], BIERBAUM J.[1], BRUNKHORST V.[1], BRUNKHORST J.[1], NIEMEIJER R.[2]

[1]Trilogy Analytical Laboratory, 870 Vossbrink Dr., Washington, MO 63090, USA; [2]R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany,

carrie@trilogylab.com; r.niemeijer@r-biopharm.de

Deoxynivalenol (DON) is a common problem in areas growing wheat and barley North America and Europe. Each year levels fluctuate from low to high. One things remains constant however, sample preparation for this commodity is critical for obtaining accurate DON results. This study evaluates sample preparation of barley containing DON. Sample preparation is a critical part of the total analytical process. The difference in sample grind size, as well as the amount of sample extracted contributes to the overall result variability. An evaluation was conducted to compare the extraction of barley naturally contaminated with DON utilizing different sample grind and different sample extraction weights. The naturally contaminated barley was ground to various mesh sizes, homogenized and various sample sizes were extracted. The extraction was performed using acetonitrile (water (84/16) with a 1 hour on Eberbach shaker. The extracts were then analyzed by LC-MS/MS. Data presented shows the effect of grind size and sample extraction size has on DON results variability.

to Index

MYCOTOXIN ANALYSES OF PERUVIAN FERMENTATIONS OF *THEOBROMA CACAO* L

NOURRISSON M.[1], BALSTER H.[1], JANSSEN K.[2], BISPING B.[1]
[1]*University of Hamburg (UHH), Hamburg School of Food Science, Department of Food Microbiology and Biotechnology, Hamburg, Germany;* [2]*Institut für Qualitätsförderung in der Süßwarenwirtschaft e.V. (IQ.Köln), Cologne, Germany.*
matthieu.nourrisson@uni-hamburg.de; bisping@uni-hamburg.de

The seeds of the cacao tree *Theobroma cacao* L. are used for the production of high quality chocolate. Raw cacao of high quality is indispensable for its production and should be free of any contaminates. This quality depends, among other things, on post-harvest treatment: fermentation and drying of cacao seeds. Misfermentation can lead to false flavors and mycotoxin contamination. Within the framework of the CORNET project "CocoaChain" funded by the FEI, samples of spontaneous cacao fermentations were taken in 2017 and 2018 at four different locations in Peru to determine the biodiversity of microorganisms and to analyze biochemical parameters of the fermented cacao beans. Alongside these analyses, mycotoxin concentrations of dried beans were checked. All fermentations were carried out in a similar way and depending on the region with or without banana leaves. Nevertheless, differences concerning the mycotoxin concentrations could be observed. In the region of Tarapoto small amounts (up to 2.1 µg/kg) of T2-toxin were detected, whereas fermented beans from Quillabamba showed no detectable mycotoxin contamination at all. In the region of Ivochote, ochratoxin A and zearaleone were detected in the beans. Zearaleone was also found in the northern region of Piura. In both regions, an increase of the zearaleone-concentration in the first 3 days of fermentation was observed, followed by an abrupt decrease. Further research is needed to understand this decline. The use of banana leaves may be expected to increase the risk of contamination with toxins from *Fusarium* sp..

Acknowledgement: We acknowledge the financial support of AiF/FEI within the project Cornet AiF 169 EN/1: "Quality improved cocoa and cocoa-based products with flavour profiles on demand – 'From farm to chocolate bar' (CocoaChain)".
Further on the authors acknowledge the support and allowance for export of genetical resources of "Instituto Nacional de Innovación Agraria – INIA" Lima, Peru.

MULTIPLE MYCOTOXINS DETECTED IN CORN SAMPLES RECEIVED BETWEEN SEPTEMBER 2019 AND FEB 2020

RAJ J.*, FARKAŠ H., JAKOVČEVIĆ Z.,
BOŠNJAK-NEUMÜLLERJ.,VASILJEVIĆ M.
PATENT CO, DOO., Vlade Ćetkovića 1A, 24 211, Mišićevo, Serbia
jog.raj@patent-co.com

The recent studies have shown that regular monitoring of feed materials for mycotoxins is important understand the risk posed by them. Therefore, the aim of this study was to screen corn samples for mycotoxins, received from different countries between September 2019 to Feb 2020. The corn samples were collected from Mexico, Peru, Columbia, Argentina, Brazil, Thailand, Taiwan, Egypt, Serbia South Africa, Spain, France, Italy, India, Vietnam, Bosnia, and Croatia. The samples were analysed by LC-MS/MS triple quad (Agilent 6460 series) based multi-mycotoxin method for quantitation of all mycotoxins (Aflatoxin B1, B2, G1 and G2, Ochratoxin A, Zearalenone (ZON), Deoxynivalenol (DON), FB1&B2, T-2 & HT-2 toxins) regulated in EU in feed by EU Directive 2002/32/EC, 2006/576/EC and 2013/165/EU. This survey concluded that 95 % of corn harvested around the world in 2019 is predominantly contaminated with Fumonisins but deoxynivalenol and zearalenone were also detected.

EUROFINS TECHNOLOGIES BRINGS RELIABLE AND SMART SCREENING SOLUTIONS TO INDUSTRIES FOR MYCOTOXINS MONITORING

ROSAR G.
Eurofins Tecna, c/o Area Science Park, Padriciano 99, 34149 Trieste
giuliarosar@eurofins.com

Immunochemistry has been exploited in the past decades to supply food and feed industries with screening devices for on-field, fast, easy and reliable verification of mycotoxins contamination. Throughput flexibility, cost effectiveness, robustness are also main parameters industries look for. Depending on the importance of each feature for each specific situation, different technologies could fit better different necessities.
The aim of the present communication is to focus on fumonisins analysis in cereals and cereals-by products. Going through the company portfolio, the performances and the features of one lateral flow device, one fast ELISA, one master-curve calibrated assay and one extra-sensitive enzyme-assay will be compared. The kits have been compared by means of reference materials run in multiples in different sessions, thus assessing both the repeatability, the intermediate reproducibility and the accuracy of each system. Time-to-result, cost per determination, measuring range and in-matrix sensitivity were also compared.

DEOXYNIVALENOL SCREENING THROUGH A NOVEL ACCURATE AND PRECISE ELISA TEST KIT

BIANCO E., BRAVIN F., ROSAR G.*, DIANA F.
Eurofins Technologies Tecna, Padriciano 99, 34149 Trieste (Italy)
Giuliarosar@eurofins.com

H2DON is a novel 20-minutes enzyme immunoassay for the quantitative detection of deoxynivalenol in the range 0.2 – 30 ppm. The assay has been validated to assess the matrix effect, the sensitivity and the trueness on maize, wheat, durum wheat, barley, (whole) oats. The Limit of Quantification (LOQ) was set at 0.3 ppm for maize, wheat and durum wheat and 0.5 ppm for barley and oats. The assay bias was investigated by means of reference materials from different suppliers. The mean recovery was 95 ± 17% (n = 78) for maize, 101 ± 17% for wheat (n = 72), 99 ± 17% for barley (n = 36) and 94 ± 8% (n = 9) for oats. Since no reference materials were available for durum wheat, the accuracy of the assay was evaluated by comparing results to LC-MS/MS analysis of incurred samples. The mean recovery was 103 ± 15% (n = 51). The assay performances on other matrices were then investigated, including DDGS, soybean meal, corn gluten meal, malted barley, swine feed, brown rice, dehulled oats. The verification of the specificity showed little to no interference. Accuracy and precision were assessed by analysing incurred and spiked materials, when no reference materials were available. High accuracy was achieved for complex and uneven matrices: the mean recovery was 87 ± 5 % (n = 45) for incurred DDGS, 99 ± 14% (n = 54) for spiked soybean meal, 90 ± 9% (n = 90) for spiked corn gluten meal, 84 ± 10% (n = 18) for reference malted barley, 97 ± 17% (n = 36) for naturally contaminated feed materials for pigs, 108 ± 15% (n = 90) for spiked brown rice and 94 ± 15% (n = 54) for spiked dehulled oats. Keeping all sample preparations easy and fast, without including any purification step was one of the main objective of this project. As a result, H2DON turns to be a reliable screening tool for rapid determination of deoxynivalenol in several matrices.

PERFORMANCE COMPARISON BETWEEN CLASSICAL AND MASTER-CURVE CALIBRATED ENZYME IMMUNOASSAYS

ROSAR G.*, FRANCESCA DIANA
Eurofins Technologies Tecna, Padriciano 99, 34149 Trieste (Italy)
Giuliarosar@eurofins.com

The cost of quality programs is a primary issue for food and feed production chain. A proper risk management must take into account which are the contaminants that could realistically occur in the materials used for production, and search for reasonable, flexible, clever, cost effective solutions leading to reliable and robust analysis. Is that possible to obtain high quality results with limited investments? B ZERO is a line of low-cost microplate enzyme immunoassays where calibrators have been physically removed. This means that, for each analytical session, the analyst is requested to run one well per sample plus one single well for a non-contaminated standard solution, so-called "zero" standard. Since no further calibrators have to be run, every session leads to a consistent savings of wells, that means that the whole kit can be used for a bigger number of samples, leading to a consistent saving for the user. The effect is dramatically important for those companies dealing with a few samples per session. Is this line of ELISA test kits reliable? The removal of the calibrators do not affect the quality of results. Quantitative data are obtained by means of a stable, batch related virtual master curve that is provided in the certificate of analysis of each kit. The interpolation of the relative signal of each sample in the master curve leads to the concentration of the material, with no relevant differences to common ELISAs where the calibration curve is run in each session. Thanks to reagents robustness, the zero standard is sufficient to standardize the results under controlled but variable laboratory conditions and guarantee reliable results. The quality of the analysis is indeed improved also by the simplification of the assay procedure, the lack of standard manipulation, mismatch, contamination or evaporation. B ZERO is a comprehensive line of kits for the quantitative detection of aflatoxin B1, aflatoxin M1, deoxynivalenol, fumonisins, ochratoxin, T2 toxin, zearalenone in many matrices. In the present work, a review of performances is shown.

QUANTITATIVE DETERMINATION OF FUMONISINS IN EGG YOLK AND EGG ALBUMEN

SCHÖNE I.R*, CZABANY T. , HARTINGER D.
BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria.
irene.schoener@biomin.net

Fumonisins are toxic fungal metabolites produced by certain *Fusarium* spp. that frequently infect maize and other agricultural crops. Although the residue levels of fumonisins (FBs) in eggs from laying hens fed with contaminated grain is negligible, carry over of partially and fully hydrolysed fumonisins (pHFBs and HFBs) is poorly investigated.

The aim of this work was to develop an analytical method for quantitative determination of FBs, pHFBs and HFBs in egg yolk and egg albumen separately. Different sample preparation procedures have been tested for the 12 analytes of interest and aliquots of the different preparation steps were analysed by LC-MS/MS: 1) A QuEChERS technique combined with a lipid removal approach, 2) protein precipitation with methanol/acetonitrile (50/50, v/v) followed by an extraction with acetonitrile/water/formic acid (74/25/1, v/v/v) and a lipid removal approach, 3) defatting with n-hexane prior extraction, 4) defatting with n-hexane during extraction and 5) defatting with n-hexane after extraction. Apparent recoveries were evaluated by spiking blank matrix prior to sample preparation at two different concentrations.

While unacceptable low recoveries could be observed with the QuEChERS technique (6-56%), protein precipitation approach resulted in recoveries between 59-100%. The most promising sample preparation method for egg yolk and albumen was a defatting step after a 2-step extraction (30 min each) with recoveries between 89-105%.

INFLUENCE OF FATTY ACID LEVELS ON FREE AND HYDROLYSED FUMONISINS AT DIFFERENT HARVEST TIMES

SHAIKH A *., VAN ZYL K., VILJOEN A., ROSE L.J.
Department of Plant Pathology, StellenboschUniversity, Private Bag X1, Matieland, 7602
ayesha@sun.ac.za

The kernel micro-environment plays a crucial role in the infection of maize by the fungus *Fusarium verticillioides* and its primary mycotoxin, fumonisins (FUM). Lipids produced within the kernel may regulate the plant defence response. Furthermore, lipids act as substrates to which fumonisins can be non-covalently linked and are termed masked/hydrolysed fumonisins. Therefore, this study aimed to investigate the potential relationship of fatty acidsproduced in four maize inbred lines resistant or susceptible to Fusarium ear rot with free and hydrolysed fumonisin (HF) contamination. Primary ears of the inbred lines were inoculated with *F. verticillioides* at 7 days after pollination, and a second set of plants were inoculated 35dap. Water-inoculated plants served as controls. Ears were harvested 7, 28, 42 and 52 days after inoculation (dai), and the free FUM and HF content was determined by liquid chromatography-mass spectrometry and fatty-acid content by gas chromatography-mass spectrometry. The amount of free FUM and HF differed significantly between maize inbred lines inoculated at both 7 and 35 dap, and increased significantly as maize kernels matured ($P<0.0001$). The free FUM content of the inbred lines were strongly correlated with HF ($R=0.84$). Conversely, no correlation was found between fatty-acids and free FUM or HF content. Understanding the role of fatty acids in fumonisin production will assist with the development of resistant maize genotypes, ultimately allowing for better management of *F. verticillioides* in the field.

to Index

DEVELOPMENT AND VALIDATION OF A MULTI-CLASS LC-MS/MS METHOD FOR BIOTOXINS AND RESIDUES IN COMPLEX FEED MATRICES: CHALLENGES AND SOLUTIONS

STEINER D.[1*], SULYOK M.[2], MALACHOVA A.1, KRSKA R.[2,3]

[1]FFoQSI – Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Head Office:FFoQSI GmbH, Technopark 1C, A – 3430 Tulln, Austria, University of Natural Resources and Life Sciences, Vienna (BOKU); [2]Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, IFA-Tulln, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria; [3]Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, UniversityRoad, Belfast, BT7 1NN, Northern Ireland, United Kingdom

david.steiner@ffoqsi.at

Influencing factors such as climate or storage conditions as well as processing of agricultural raw materials allow a variety of contaminants like fungal metabolites or plant toxins to enter the food and feed chain. Additionally, the impact of environmental chemical pollution has steadily increased within the last decades by the excessive use of pesticides and pharmacological active agents. These so-called emerging contaminants become a new environmental problem, since there is still a lack of knowledge about long-term risks for non-target organisms. Consequently, there is a growing need for robust, reliable and comprehensive analytical methods, which allow a sensitive, selective and rapid determination of such naturally and anthropogenic pollutants in environmental samples. In this work, a liquid chromatography-electrospray ionization tandem mass spectrometric method was developed and validated to allow a simultaneous quantification of about 700 fungal metabolites, 500 pesticides, 150 veterinary drugs and 50 plant toxins. The aim of this work is a demonstration of the applicability of a generic extraction method for more than 1,400 analytes from different substance classes in complex animal feedstuff. Further focus is on the assumption that significant differences in signal suppression and enhancement (SSE) as well as in the extraction efficiency (RE) within different lots of a sample type is substantially influencing the method performance. In order to perform a comprehensive characterization and assignment of corresponding extraction losses and matrix effects,12 main single feed ingredients of complex compound feed rations for cattle, pig and chicken were investigated. For validation purposes, artificial complex model matrices, composed of blank individual components, were prepared in-house and the related numerical values for signal suppression/enhancement were modelled based on the data derived from the individual single feed ingredients. Comparability between model and real samples was statistically tested, by comparing 7 different replicates for each matrix type. High matrix effects, absolute and relative, were revealed as main negative contributor to the overall analytical performance. However, model matrices were less prone to influences of sample inhomogeneity, due to the reduced natural background contamination. Insummary the work presents a fit-for-purpose validation proposal for LC-MS/MS multi-class approachesin complex compound feed matrices.

The competence centre FFoQSI is funded by the Austrian ministries BMVIT, BMWFW and the Austrian provinces Niederoesterreich, Upper Austria and Vienna within the scope of COMET -Competence Centers for Excellent Technologies. The programme COMET is handled by the Austrian Research Promotion Agency FFG.

DIAGNOSTIC FRAGMENT FILTERING FOR UNRAVELLING THE EGOT ALKALOID AND INDOLE–DITERPENOID METABOLOME IN *CLAVICEPS PURPUREA SENSU LATO*

UHLIG S.[1*], RANGEL-HUERTA O.D.[1], PAUCHON K.[2], ROLEN E.[1], DIVON H.[1], SUMARAH M.[3], VRÅLSTAD T.[1], RENAUD J.[3]

[1]Norwegian Veterinary Institute, Ullevålsveien 68, 0454 Oslo, Norway. [2]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Science, Universitetstunet 3, 1430 Ås, Norway. [3]London Research and Development Center, Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada.

silvio.uhlig@vetinst.no

The grass parasitic fungus *Claviceps purpurea sensu lato* produces sclerotia with toxic ergot alkaloids and uncharacterized indole–diterpenoids. The aim of this study was to tentatively identify as many peptide ergot alkaloids and indole–diterpenoids as possible by using liquid chromatography high-resolution mass spectrometry (LC–HRMS), and the built-in diagnostic fragment filtering tool in MZmine 2 for data extraction. The sample set consisted of 66 *Claviceps* sclerotia from four different geographic locations in Southeastern Norway as well as Saskatchewan, Canada. The host plants included both wild grasses and important cereal grains such as rye. DNA sequencing showed that the sclerotia were from three *Claviceps* species, i.e. *C. purpurea sensu stricto*, *C. humidiphila* and *C. arundinis* (former *C. purpurea* genotypes G1, G2 and G2a, respectively). All sclerotia from cereal grains were from *C. purpurea sensu stricto*. Diagnostic fragment filtering was based on selected MS/MS product-ions that are well conserved across the different ergot alkaloid subgroups and indole–diterpenoids of the paspaline type. The approach extracted mass spectra from 67 peptide ergot alkaloids (including C-8 epimers and lactam variants) and five indole–diterpenoids. In addition, three clavines were detected using targeted analysis. In several samples, the sum of the peak areas for ergot alkaloids, which have been assigned as "major" analogues by EFSA, accounted only for ca. 50% of the extracted total ergot alkaloid metabolome. Multivariate statistical analyses showed that several of the alkaloids are specific for certain species within the *C. purpurea* species complex and could be used as chemical markers for species assignment.

This research was financially supported by the Norwegian Research Council (Strategic Institutional Program "FUNtox")

RAPID ON-SITE SIMULTANEOUS DETERMINATION FOR MULTIPLE MYCOTOXINS AND PESTICIDES IN AGRO-FOOD

ZHANG W.[1], ZHANG Q.[1], ZHANG Z.[1], TANG X.[1], LI P.[1]
[1]*National Reference Laboratory for Agricultural Testing P.R.China, Key Laboratory of Detection for Mycotoxins (Ministry of Agriculture), Laboratory of Quality & Safety Risk Assessment for Oilseed Products (Ministry of Agriculture),Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.*
zhangwen@oilcrops.cn

Mycotoxins and pesticides usually exist in cereal food simultaneously. Due to the different structure of these two kinds of contaminations, it is difficult to detect them simultaneously in rapid assay. In order to overcome the problem, we develop a rapid, on-site, and quantitative paper sensor to detect carbamates and aflatoxins at the same time. Two novel monoclonal antibodies (mAbs) against carbaryl and carbofuran (1D2 and G11) were prepared. The IC50 values (half maximal inhibitory concentration) were 0.8 ng/mL and 217.6 ng/mL for carbaryl and carbofuran, respectively. Based on the sensitive and specific mAbs, a multi-TRFICA (time-resolved fluorescence) paper sensor was developed, which can simultaneously detecte six types of hazardous chemicals, including AFB1, AFB2, AFG1, AFG2, carbaryl, and carbofuran. In addition, a facile sample pretreatment method formycotoxins and pesticides was explored to establish competitive indirect enzyme -linked immunosorbent assay and multi-TRFICA-paper sensor. The paper sensor can be easily observed with naked eyes, qualitatively under a UV lamp, and quantitated using a home-made device. The calculated limit of quantity for AFTs, carbaryl, and carbofuran are 0.03, 0.02, and 60.2 ng/mL in corn samples, respectively. The spiking-recoveries and real sample studies proved that multi-TRFICA-paper sensor is an accurate, sensitive, and high throughput detection method for simple and low-cost analysis in corn samples.

This research was financially supported by the National Key Research and Development Program (2016YFE0119900), Hubei Natural Science foundation (2017CFB421), and the Project of Shanghai Science and Technology Committee (17391901200).

to Index

Session 3

Prevention of mycotoxigenic fungi in the field

DISEASE MANAGEMENT PRINCIPLES TO MINIMIZE MYCOTOXIN RISK IN THE FIELD

MUNKVOLD G.P. AND MAYFIELD D.A.
Iowa State University Department of Plant Pathology & Microbiology and Seed Science Center, 2115 Osborn Dr., Ames, IA 50011, USA
munkvold@iastate.edu

Mycotoxins are a serious risk in maize produced for human food, animal feed, or biofuel. Mycotoxigenic fungi are adapted to every climate where maize is grown, and climate change promises to disrupt the geographic distribution and worsen the severity of mycotoxin problems in maize. In a MycoKey Nominal Group exercise conducted in 2018, genetic resistance, insect management, biological control, and various cultural practices were identified as effective tactics for preventing contamination by aflatoxins, fumonisins, DON, or zearalenone. Research suggests fungicide applications or seed treatments also may have some potential. Most mycotoxin contamination involves more than one compound, so it also is important to understand how management affects emerging mycotoxins. Our research on *Fusarium* spp. in the *F. fujikuroi* complex focuses on the role of insect management in prevention of several mycotoxins. In field trials in Iowa, inoculations with *F. subglutinans* or *F. temperatum* were combined with infestations of European corn borer or corn earworm on *Bt* and non-*Bt* maize hybrids. *Bt* maize was consistently lower in fumonisins, but beauvericin, moniliformin, fusaproliferin, and fusaric acid were not affected by insect infestations or maize hybrid in plots inoculated with *F. subglutinans* or *F. temperatum*. However, inoculated treatments were significantly lower in fumonisins from naturally occurring infection, suggesting that *F. subglutinans* and *F. temperatum* effectively competed against endemic fumonisin-producing *Fusarium* spp. These results provide insights about the roles of insect management and fungal competition in a holistic approach that integrates multiple tactics to provide the most effective risk reduction for mycotoxins in maize.

to Index

VALIDATION OF LESS SUSCEPTIBLE DURUM WHEAT VARIETIES TO FUSARIUM DISEASES AND TOXINS ACCUMULATION

BASSI R.
Syngenta AG.- Seed Development Cereals – South Europe

10 varieties of durum wheat in last part of stage of development according the agronomical adaptation in the Mediterranean environment, has been tested in field conditions along 3 years, to assess the sensitivity to the fusarium head blight (FHB) and the toxins (DON) accumulation as phenotypical expression. The varieties selected for their improved genetics concerning yield level and the quality requested by pasta industry, has been compared in 2 different locations in northern and center Italy with natural and artificial inoculum of different fusarium species among other relevant varieties already in the market, included the standard varieties Saragolla and Sy Cysco as sensitive and tolerant references to FHB. The test fields were managed in untreated condition with small plots using RCB design with 4 replications using farmer standard agrotechnical inputs. During the autumn-summer cycle, all the varieties has been assessed for tolerance to FHB and other diseases, vegetation behavior, yield and main quality parameters. On grains after harvest has been measured the DON level. Among the different varieties, SY 515006, Fuego and SY 516090, showed a significative tolerance in field to FHB and a stable reduction of DON accumulation close to 20-35% compared to sensitive varieties in natural conditions, linked to high yield and overall good performance. These varieties can represent a novel and clear advantage in some specific cultivation environments to be used as rough matter for pasta industry processing, to reduce the toxins level risk in end user products. Moreover, the same selected and proved genetics, can give solid bases to be applied for the parental lines identification for new crosses for future improved tolerant varieties availability.

COMBINATION OF MEASURES TO REDUCE TOXIN CONTAMINATION AT CRITICAL POINTS OF FOOD CHAIN IN CEREAL FIELDS

MESTERHAZY A.[1], LEHOCZKI-KRSJAK S.[1], VARGA M.[3], SZABÓ B.[1], BERÉNYI A.[2], AND TÓTH B.[2].

[1]*Cereal Research non-profit Ltd, 7601 Szeged Hungary.* [2] *NAIK Field Crops departement, 6726 Szeged, Hungary* [3] *Univ. Szeged, Department of Microbiology, 6726 Szeged, Hungary*

. akos.mesterhazy@ga bonakutato.hu

The toxin contamination following epidemics by toxigenic fungi is a global problem. The recent epidemics around the globe show clearly that the toxin contamination depends mostly from weather and previous crop indicating the vulnerability of the whole production chain. After screening thousands of genotypes highly significant differences between registered cultivars and advanced lines were found. This is valid for all cereal species. In a two years test it is possible to identify the plus variants. The variation is normally 10-20 fold, so this provides the most rapid way to decrease toxin exposition. The resistance is an independent variable, but the fungicide application definitely not, its effect depends on resistance level. In a highly susceptible cultivar 3.4 time higher DON can remain after treatment (40-50 % reduction), the same fungicide resulted in 0.1 mg/kg (98% reduction). The higher resistance lessens the negative effect of poor previous crop, helps to balance tillage mistakes and makes possible longer active plant life with higher and healthier yield. The harvested grain has better outlook when originated from higher resistant cultivars. The grains with similar toxin contamination should be stored grains are pooled in different storage facilities. A computer aided supervising system must work in each silo and must show real time data and alarm when a disorder arises. Toxin control should be before, during and after storage, too. The skeleton of the chain is ready, but a lot of research work is necessary to fill up the apps to be able advice farmers.

This research was financially supported by the Projects TUDFO/5157/2019/ITM 2019-2023, MycoRed FP7 (KBBE-2007-2-5-05) 2009-2012, GINOP-2.2.1-15-2016-00021, 2016-2020 and initiated by MycoKey2016-2020..

FUNGAL VOLATILE ORGANIC COMPOUNDS, CAN BE USED TO DEVELOP AFLATOXIN-SPECIFIC SENSORS

JOSSELIN L. [1A], DE CLERCK C. [1B,C], DE BOEVRE M. [2], MORETTI A. [3], SOYEURT H. [1D] AND FAUCONNIER ML. [1A]

1Gembloux Agro-Bio Tech, Liege University, Belgium
a Laboratory of Chemistry of Natural Molecules
b Integrated and Urban Plant Pathology Laboratory
c Agriculture Is Life
d Statistic, Informatic and applied Modelling (SIMa)
2Faculty of Pharmaceutical Sciences, University of Ghent, Belgium
3Italian National Research Council, Institute of Sciences of Food Production ISPA, Bari, Italy

Foodstuff (corn, wheat, rice, etc.) can be contaminated by several filamentous fungal species in pre or post-harvest conditions. Some of these, such as *Aspergillus*, *Fusarium* and *Penicillium* produce secondary metabolites, highly toxic at low concentrations to all vertebrates including humans: they can cause severe illnesses upon chronic exposure and can even lead to death after acute exposure. These non-volatile molecules are named mycotoxins and current methods to detect them, involving the use of ELISA tests or HPLC, are quite time consuming and expensive. At present there is no rapid test that does not require extensive sample preparation to detect the presence of mycotoxin directly in a production line (e.g. grain storage companies).

Therefore, the aim of this work is to identify volatile organic compounds (VOCs) emitted specifically when mycotoxins are produced that could be used as markers to detect mycotoxins in foodstuff.

Using Solid Phase Micro Extraction technique, we have characterized and compared the VOCs produced by non-toxigenic and toxigenic strains of *Aspergillus flavus* (producing aflatoxins B1). The analyses have shown similarities and differences between the two categories of strains. Both of them emit a common VOCs consisting mainly in alcohol (2-methylbutan-1-ol, propan-1-ol, 2-methylpropan-1-ol), ester (ethyl isobutyrate, ethyl acetate) and aldehyde (2-methylbutanal, 2- methyl-2-enal) many of which are known in the literature to be specific of fungi.

The most important difference a higher emission of terpenes (epizonaren, α-gurjunene, β- elemene) emitted by toxigenic strains.

A combination of potential biomarkers has been identified for the development of the future molecular fingerprint sensor.

The next step is to study the VOCs when the fungi grow on the stored grain according to the parameters related to the in vivo conditions. And confirm the correlation between specific VOCs and mycotoxin production.

EXPLORING SECONDARY METABOLITES AND *TRITICUM* SPP. BIODIVERSITY IN RELATION TO *FUSARIUM* MYCOTOXIN ACCUMULATION AND MODIFICATION

RIGHETTI L.[1], GOZZI M.[1], PEDRAZZANI[1], BRUNI R.[1], BLANDINO M.[2], DALL'ASTA C[1*].

[1]*Department of Food and Drug, University of Parma, Viale delle Scienze 27/A, 43124 Parma, Italy.*
[2]*University of Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo Braccini 2, 10095 Grugliasco, TO, Italy.*
chiara.dallasta@unipr.it

Along with genetic approaches, metabolomic fingerprint of Triticum spp. can provide powerful opportunities for plant breeding through the identification of the chemical defence pathways activated in plants. In this framework, mapping the chemical biodiversity of Triticum spp. niche varieties in response to Fusarium infection, may offer valuable insights into the frontline machinery of plant resistance.

As already reported [1] secondary metabolites such as flavonoids and phenolic acids, have been suggested to counteract toxigenic *Fusaria* and mycotoxin accumulation in wheat, although anthocyanin have not been considered yet. However, little is known so far about the role of other phenolic compounds, i.e. anthocyanins and alkylresorcinols, both located in the outer aleurone layers and involved in the plant defence frontline.

Within the available germplasm, pigmented wheat (*Triticum aestivum* L.) are characterised by the selective location of anthocyanins in the pericarp or in the outer aleurone layers, giving the grain characteristic colours (blue, black, purple or red). Overall, the accumulation of these pigments in the outer aleurone layer is considered a recently evolved trait, resulting from environmental adaptation. Their possible relation with FHB resistance was described in barley [2, 3] and wheat [4].

We have therefore investigated the potential correlation between alkylresorcinol and anthocyanins composition in wheat and multiple Fusarium-related mycotoxins accumulation, focusing on a collection of *Triticum* spp. *niche* varieties, observed over two harvest years.

THE ROLE OF FUNGAL ENDOPHYTES AS BIOLOGICAL CONTROL AGENTS AGAINST *FUSARIUM GRAMINEARUM* AND ITS MYCOTOXINS: RESULTS FROM WP4 OF THE MYCOKEY PROJECT

ABDALLAH MF.[1,2], AUDENAERT K.[2], LANDSCHOOT S.[2], DE BOEVRE M.[1], HAESAERT G.[2], AND DE SAEGER S[1].

[1]*Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Ghent, Belgium.*
[2]*Laboratory of Applied Mycology and Phenomics, Ghent University, Ghent, Belgium.*
E-mail: mohamed.fathi@ugent.be

Fusarium graminearum is a predominant fungal pathogen of cereals which also produces toxic secondary metabolites such as deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON) and zearalenone (ZEN) [1]. The potential of two endophytic fungi: *Epicoccum nigrum* (EG) and *Sordaria fimicola* (SF) to inhibit the growth of *F. graminearum* was investigated [2]. Furthermore, we investigated the effect of these endophytes on the levels of the most commonly produced mycotoxins, DON, 15-ADON and ZEN. In *in vitro* assays, the selected endophytic fungi were able to reduce both trichothecenes (DON and 15-ADON) and fungal growth via direct competition. Remarkably, these endophytes were also able to display their biocontrol traits even when there was no direct contact with the pathogen which points to the involvement of biogenic volatile compounds (BVOCs). Although all endophytes displayed a variable level of biocontrol in direct contact experiments, none of them convincingly resulted in a reduction of ZEN. However, when *F. graminearum* was exposed to BVOCs produced by the endophytes, a clear reduction in ZEN was observed which highlights the great potential of BVOCs as new agrochemical agents. Exposure of *F. graminearum* to the well-known biocontrol agents *Trichoderma harzianum* spp. and *Clonostachys rosea* spp. did not result in ZEN reduction. Finally, biocontrol traits were validated in an *in planta* assay using a maize -*F. graminearum* model system. In maize plants, a clear reduction of ZEN, DON, 15-ADON in addition to the modified mycotoxin deoxynivalenol-3-glucoside levels was observed in the presence of the fungal endophytes.

This work was supported by the MYCOKEY project which is funded by the Horizon 2020 research and innovation programme under the grant agreement No. 678781. The authors are also thankful to Hercules Foundation project AUG/13/13.

to Index

EFFECT OF BRASSICA TISSUES ON MYCOTOXIN-PRODUCER *FUSARIUM GRAMINEARUM*

S. ASHIQ, A. WATSON, S. EDWARDS AND M. BACK
Harper Adams University, Newport, Shropshire, TF10 8NB, UK.
sashiq@harper-adams.ac.uk

The *Fusarium* genus is a producer of the largest and most diverse group of mycotoxins, which occur abundantly in cereals. The globally important cereal pathogen, *Fusarium graminearum* causes head blight in wheat, resulting in yield losses and mycotoxin contamination. *Fusarium graminearum* produces the mycotoxins deoxynivalenol and zearalenone. Currently, triazole fungicides are used to suppress *Fusarium graminearum*, however, limited effectiveness of triazoles and concerns over safety of pesticides have led to the pursuit of alternatives such as biofumigation. Biofumigation involves growing short term brassica crops, followed by maceration of the plant tissue and rapid incorporation into the soil. Inhibitory substances, particularly isothiocyanates, are released as a result of damage to brassica plant tissue causing suppression of soil borne pests and diseases. The application of biofumigant brassica crops, as an alternative crop protection method for soil borne pathogens and pests is increasingly gaining interest. However, research on the potential of biofumigation to reduce the inoculum of *Fusarium* species affecting cereals is scarce. The effect of volatile compounds from damaged brassica tissues on *Fusarium graminearum* growth was assessed in multiple laboratory assays. *Fusarium graminearum* cultures were exposed to leaf discs of brassicas, such as Indian mustard, oilseed radish and rocket. Indian mustard (*Brassica juncea*) leaf discs were effective against mycelial growth showing up to 100% suppression, while the sinigrin content in the leaf tissue corresponded with the level of suppression. Findings suggest that brassica plants could have suppressive effect on reducing the inoculum of *Fusarium graminearum* in soil prior to cereal production.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678012.

FREE-AIR CO_2 ENRICHMENT (FACE) IMPACT ON *FUSARIUM* MYCOTOXINS AND THEIR MYCOTOXIGENIC PRODUCER FUNGI

SCARPINO V.[1], BADECK F.[2], TERZI V.[2], RIZZA F.[2], MORCIA C.[2], GHIZZONI R.[2] AND BLANDINO M.[1].

[1]Università degli Studi di Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, 10095 Grugliasco (TO), Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Research Centre for Genomics and Bioinformatics, via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy.

massimo.blandino@unito.it

The effect of elevated CO_2 (e[CO_2], 570 ppm) compared to ambient (a[CO_2] = 404 ppm) on *Fusarium* mycotoxin contamination in winter cereals (wheat and oat) and their mycotoxigenic producer fungi was investigated with FACE experiments. The comparison between e[CO_2] and a[CO_2] was carried out in 3 different growing seasons (2011–12, 2012–13 and 2015–16) and different genotypes (2 bread wheat, 2 durum wheat and 2 oat cultivars).

On average, the genotypes significantly differed in terms of their mycotoxin content. Common wheats had the lowest levels of deoxynivalenol, their modified forms and emerging mycotoxins. The highest deoxynivalenol content was recorded in durum wheat, while oats resulted more prone to T2/HT2 toxins, enniatins and nivalenol. In all years, e[CO_2] significantly increased all the aforementioned mycotoxins, from 1.4 to 2.8 times.

The fungal DNA content of grains did not differ significantly between e[CO_2] and a[CO_2] for none of the cvs in all years. Oats were more susceptible to colonisation by *F. langsethiae* and *F. poae*, durum wheats by *F. culmorum/graminearum* and *F. avenaceum*. Across all samples colonisation with *F. langsethiae* and *F. poae* were positively correlated (50% covariation). Correlations between the remaining pairs of colonisers were weakly positive or non-significant suggesting absence of mutual exclusion between the fungal species. Across all samples *F. langsethiae* and T2/HT2 toxins, *F. poae* and nivalenol as well as *F. avenaceum* and enniatins were positively correlated with covariation of ≥50%.

The results indicate that future rising CO_2 levels, may increase the threat of grain mycotoxins contamination, as a consequence of a higher toxinogenesis of fungal producers.

This research was financially supported by the Project Duco by the "Fondazione in rete per la ricerca agroalimentare" within the AGER program and DiBio–BIOPRIME project (Prot. 76381, MiPAAF PQAI I), funded by Italian Ministry of Agriculture and Forestry.

BACILLUS VELEZENSIS RC 218 AS A SUCCESSFUL BIOCONTROL AGENT TO REDUCE FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION ENSURING FOOD QUALITY PARAMETERS ON HARVESTED WHEAT

CANTORO R.[1], PALAZZINI J.M.[1], YERKOVICH N.[1], DUNLAP C.[2], MIR L.R.[3], CHIOTTA M.L.[1], TORRES A. [1], RAMIREZ M.L.[1] AND CHULZE S.[1].

[1]Research Institute on Mycology and Mycotoxicology (IMICO), The National Scientific and Technical Research Council (CONICET)- *National University of Río Cuarto- (UNRC),* (5800) *Río Cuarto-Córdoba, Argentina. [2] USDA, Peoria Illinois, USA. [3]Wheat and Soybean Quality Laboratory, National Institute of Agriculture Research, INTA, Córdoba, Argentina.*

schulze@exa.unrc.edu.ar

Fusarium head blight (FHB) is one of the main fungal diseases affecting bread (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.) crops worldwide, resulting in important economic losses such as reduced grain yield, quality and safety due to mycotoxin contamination, mainly deoxynivalenol (DON). In Argentina, FHB is caused mainly by *Fusarium graminearum sensu stricto* when humid weather conditions prevail during anthesis stage. Management strategies commonly used to cope with FHB are not highly efficient if individually applied. Biocontrol appears as an environmentally friendly tool that can be incorporated within an integrated management program. Previous studies carried out by our research group highlighted the effectiveness of *Bacillus velezensis* RC 218 to reduce FHB severity and DON accumulation. The aim of the present study was to evaluate the biocontrol activity of *B. velezensis* RC 218 during 2019 harvest season in Córdoba Province, Argentina on bread wheat infected by *F. graminearum ss*. At anthesis the pathogen and the biocontrol agent were applied. After 21 days, disease incidence and severity were evaluated. At harvest maturity, quality parameters and DON accumulation were analyzed. *B. velezensis* caused reductions of 37.5% and 33.3% in disease severity and *Fusarium*-damaged kernels respectively. A significant reduction in DON accumulation was observed. Regarding quality parameters flour ash values and alveograph parameters were improved in the treatments with the biocontrol agent. Microbiome dynamic preliminary data from wheat spikes treated with the pathogen, biocontrol agent and control treatments were also evaluated.

This research was financially supported by the European Union's Horizon 2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey).

FUSARIUM GRAMINEARUM SENSU STRICTO AND FUSARIUM POAE ISOLATED FROM DIFFERENT BARLEY REGIONS IN ARGENTINA AND BIOCONTROL IN VITRO, UNDER FIELD CONDITIONS AND DURING THE MICROMALTING PROCESS

CHIOTTA M.L.[1], ALANIZ ZANON M.S.[1], AGUINAGA A.[2], GRIBALDO A.[2], YERKOVICH N.[1], CANTORO R.[1], PALACIOS S.[1], PALAZZINI J.[1], SULYOK M.[3], PUNTILLO M.[4], VINDEROLA G.[4], DUNLAP C.[5], CHULZE S.N [1].

[1]Research Institute on Mycology and Mycotoxicology (IMICO), National University of Río Cuarto-(UNRC), The National Scientific and Technical Research Council (CONICET), (5800) *Río Cuarto-Córdoba, Argentina. [2]Cerveceria y Malteria Quilmes, Anheuser-Busch InBev. Tres Arroyos, Buenos Aires, Argentina. [3]Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna, Tulln, Austria. [4]Instituto de Lactologia Industrial (INLAIN) CONICET-UNL, Santa Fé, Argentina. [5]USDA, Peoria Illinois, USA.*

schulze@exa.unrc.edu.ar

Barley (*Hordeum vulgare* L.) is the second most important winter crop after wheat in Argentina, and is mainly used for the malt production in beer manufacture. *Fusarium* species, mainly *Fusarium graminearum* sensu stricto and *F. poae*, cause *Fusarium* Head Blight (FHB) in barley and produce reductions in quality and safety pre-harvest and during the malting process due to fungal growth and mycotoxin contamination. The aims of the present study were- to evaluate incidence of *F. graminearum* species complex and *F. poae* in different barley-growing regions from Argentina, - to determine their toxigenic ability, -to evaluate the biocontrol activity of *B. velezensis* RC218 to reduce *F. graminearum ss* and DON accumulation under field conditions and during micromalting process –to select new potential biocontrol agents to control *F. graminearum* ss and *F. poae* growth under in vitro conditions. Data showed that *F. graminearum* ss was isolated with high incidence in all regions during 2016 and 2017 harvest seasons. The chemotype of the strains was DON-15ADON and DON-3ADON, also the strains were producers of ZEA and their derivatives. NX-2 chemotype was detected for the first time from strains isolated from barley in Argentina. *Fusarium poae* was isolated in two regions and were NIV producers, besides other toxins produced by the strains were DAS, NEO, MAS and T2-tetraol. The application of *B. velezensis* RC218 *in vitro*, under field conditions and during micromalting process showed reduction of *F. graminearum* ss fungal growth and DON accumulation. Some new potential biocontrol agents were selected such as *Lactobacillus plantarum* and *B. subtillis* subsp. *inaquasorum.*

This research was financially supported by the EU Project MycoKey N. 678781 (http://www.mycokey.eu/).

DEVELOPMENT OF AN INTEGRATED AND OPEN SOURCE WORKFLOW FOR LC-HRMS METABOLOMICS STUDIES. CASE STUDY: METABOLIC CHANGES OF MAIZE IN RESPONSE TO FUSARIUM VERTICILLIOIDES INFECTION

CIASCA B.[1]*, LATTANZIO V.M.[1]*, LANUBILE A.[2], MAROCCO A.[2], PASCALE M.[1], LOGRIECO A.F.[1]

[1]*Institute of Sciences of Food Production, National Research Council, Via Amendola, 122/O, 70126 Bari, Italy* [2]*Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122, Piacenza, Italy*

biancamaria.ciasca@ispa.cnr.it

Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) represents the most powerful metabolomics platform to investigate biological systems. Reproducible and standardized workflows allow to obtain a meaningful biological interpretation. The purpose of this study was to set up and apply an open source workflow for LC-HRMS metabolomics studies. Key steps of the proposed workflow were: (1) experimental design, (2) sample preparation, (3) LC-HRMS analysis, (4) data processing, (5) custom database search, (6) statistical analysis, (7) compound identification and (8) biochemical interpretation. Its applicability was evaluated through the study of metabolomics changes of two maize recombinant inbred lines (RIL) with contrasting phenotypes with respect to disease severity after *F. verticillioides* infection of seedlings. Analysis of data from the case study revealed abundance change in metabolites belonging to different metabolic pathways, including two amino acids (L-tryptophan and tyrosine), three N-hydroxynnamic acid amides (HCAAs) and four flavonoids.

INNOVATIVE CROPPING SYSTEMS AND BIOPESTICIDES TO PREVENT MYCOTOXINS IN WHEAT

DRAKOPOULOS D.[1,2], KÄGI A.[1], SIX J.[2], MECA G.[3], GIMENO A.[1], JENNY E.[1], BÄNZIGER I.[1], FORRER H.R.[1], MUSA T.[1] AND VOGELGSANG S.[1].

[1]Agroscope, 8046 Zurich, Switzerland; [2]ETH Zurich, 8092 Zurich, Switzerland; [3]University of Valencia, 46100 Burjassot, Spain.

susanne.vogelgsang@agroscope.admin.ch

Fusarium head blight (FHB) is a devastating fungal disease of wheat worldwide causing yield losses and grain contaminations with hazardous mycotoxins, such as deoxynivalenol (DON). In maize-wheat rotations with reduced or no-tillage, the remaining maize crop residues on the soil surface represent an important inoculum source for FHB infection of the subsequent cereal crop. The overall objective of this project was to explore prevention strategies in the field to reduce FHB infection and mycotoxins in wheat under minimum tillage. We investigated the following strategies: (a) application of cut-and-carry biofumigation and botanicals on maize crop residues (Drakopoulos et al., 2019; Drakopoulos et al., 2020); (b) maize-intercropping; and (c) cover cropping. The use of cut-and-carry biofumigation with mulch layers from white mustard, Indian mustard or berseem clover reduced the DON content in wheat grain by up to 58 %, whereas the majority of the tested botanicals did not show a consistent efficacy throughout the experimental years. The intercropping of white mustard or Indian mustard with grain maize decreased the DON content in wheat grain by 52 % or 32 %, respectively, compared with maize as a sole crop. Cover cropping of white mustard, Indian mustard or winter pea after the harvest of silage maize resulted in lower DON contents and higher crop yields of spring wheat compared with the herbicide treatment without growing a cover crop. Within the context of sustainable crop protection strategies, cereal growers could benefit from the recommended prevention measures by improving safety and yield of wheat grain.

This research was supported by the project MycoKey "Integrated and innovative key actions for mycotoxin management in the food and feed chain", Horizon 2020, grant no. 678781 and the Swiss State Secretariat for Education, Research and Innovation.

to Index

FUNGICIDE AND BIOPESTICIDE CONTROL OF FUSARIUM HEAD BLIGHT

EDWARDS S.G.[1] AND HOFGAARD I.[2].
[1]*Harper Adams University, Newport, Shropshire, TF10 8NB UK.* [2]*Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway.*
sedwards@harper-adams.ac.uk

Chemical control of Fusarium head blight (FHB) is currently limited to the triazole fungicides. This is problematic due to the high selection pressure for fungicide resistance and current safety concerns for some triazole fungicides. Consequently, there is a growing interest in the use of alternative products to control FHB. The term biopesticide covers a wide spectrum of potential products used within plant protection and includes simple salts, plant defence elicitors, biological control agents and botanical extracts. As part of the MyToolBox project, field experiments were conducted for wheat in the UK and oats in Norway on the efficacy of a range of old and new chemistry fungicides and biopesticides to control FHB and consequently reduce concentrations of deoxynivalenol (DON) in harvested grain. In general, old chemistry fungicides had limited efficacy to control FHB and DON whilst the biopesticides tested failed to demonstrate any activity towards FHB and DON. A new SDHI fungicide, Adepidyn™ developed by Syngenta was found to be highly effective at reducing FHB and DON. Once registered, this product will prove an important addition to the chemical control of FHB and DON contamination of cereals.

The MyToolBox project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678012.From 2007 to 2009

IN VITRO EVALUATION OF COMMERCIAL FUNGICIDES AGAINST MYCOTOXIGENIC *FUSARIUM* AND *ALTERNARIA* SPECIES ASSOCIATED WITH WHEAT IN SOUTH AFRICA

JACOBS C.M.[1], VAN COLLER G.V.[2], VILJOEN A.[1] AND ROSE L.J[1].
[1]*Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602;*
[2]*Western Cape Department of Agriculture, Elsenburg 7607, South Africa.*
carlynn@sun.ac.za

Wheat grain in South Africa is affected by mycotoxigenic fungi, including species in the genera *Fusarium* and *Alternaria*. Infection results in reduced grain yield and quality and the contamination of grain with mycotoxins. The purpose of this study, therefore, was to evaluate the effect of commercial fungicides registered for foliar diseases of wheat, for the management of *Fusarium* spp. and *A. alternata* associated with grain in South Africa. Fungicides containing tebuconazole, tebuconazole + prothioconazole and epoxiconazole + pyraclostrobin were tested *in vitro* using assays where i) agar was amended with fungicide, ii) fungicide was distributed on agar and iii) a disk diffusion assay. The concentrations of active ingredients ranged from 5-5000 ppm. The percentage inhibition (%Inb) was calculated for each isolate and used to determine $EC_{50, 80, 90}$ values. *Alternaria alternata* had the highest mean $EC_{50, 80, 90}$ values for all the fungicides tested when evaluated on fungicide-amended media. Concentrations ranged from 18.3-924 692 ppm for tebuconazole, 8.0-311.7 ppm for tebuconazole + prothioconazole, and 8.2–1136.9 ppm for epoxiconazole + pyraclostrobin. Similarly, *A. alternata* was the least sensitive to tebuconazole and tebuconazole + prothioconazole when evaluated using the other two assays. The *Fusarium* species were more sensitive to all fungicides with lower %Inb mean value and $EC_{50, 80, 90}$ variables when evaluated on amended media. However, *Fusarium graminearum* was the least sensitive species with higher $EC_{50, 80, 90}$ values for epoxiconazole + pyraclostrobin when evaluated using the disk diffusion assay. These results indicate the potential of foliar fungicides to contribute to the management of mycotoxigenic fungi associated with wheat grain.

This research was financially supported by South Africa's National Research Foundation (NRF).

to Index

AN INTEGRATED APPROACH TO MANAGE MYCOTOXIN CONTAMINATION IN SOUTHERN AFRICAN MAIZE GRAIN

JANSE VAN RENSBURG B.[1], FLETT B.C.[1], ROSE L.J[2] AND MABUZA L.M.[1]

[1]Agricultural Research Council – Grain Crops, Chris Hani street 114, Potchefstroom, 2530, South Africa. [2]Stellenbosch University – Department of Plant Pathology, Lombardi Building, Corner of Victoria and Neethling street, Stellenbosch, South Africa.

BelindaJ@arc.agric.za

Disease and mycotoxin management strategies for maize include tillage practices and crop rotations which have not been sufficiently evaluated in South Africa. The first aim of this study, was to establish the effect of cropping systems on the infection of mycotoxigenic fungi and mycotoxin production in maize grain. Certain cropping systems with an increase in maize residue, can increase the primary inoculum of foliar diseases. The second aim of this study was to establish the effect of prophylactic foliar fungicide applications on the infection of mycotoxigenic *Fusarium* spp. and mycotoxin production in maize grain. The effect of cropping systems and prophylactic fungicide spray regimes on ear rot infections and mycotoxin accumulation was investigated in Erfdeel (2011 – 2015) and Buffelsvallei (2009 – 2015) as well as Cedara, Potchefstroom and Vaalharts (2017-2019), respectively. Four spray combinations were administered at five different plant growth stages with different time intervals. Mycotoxin analyses and target DNA quantifications in both studies were conducted with HPLC and qPCR. Disease incidence and mycotoxin contamination at Buffelsvallei were inconsistent and not significant at Erfdeel. In the prophylactic fungicide spray trial, infection of maize grain by *F. verticillioides* and *F. boothii* varied over localities. None of the mycotoxin levels exceeded South African regulatory limits. Spray combinations containing Azoxystrobin 200g/l + Difenoconazole 125g/l was 50% less effective in reducing fungal infection compared to the other spray combinations irrespective of application dates. Evaluated CA systems, did not increase the risk of maize ear rots and mycotoxin production. Certain fungicide spray combinations can reduce maize ear rots.

This research was financially supported by the Maize Trust of South Africa Project MTM16/02

GENETIC TOOLS FOR BREEDING MAIZE RESISTANT TO *FUSARIUM VERTICILLIOIDES*

MAROCCO A., LANUBILE A., STAGNATI L.
Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.
E-mail: adriano.marocco@unicatt.it.

Fungal infection by *Fusarium verticillioides* is cause of substantial reductions in maize yield and grain quality worldwide. Developing natural resistance in maize genotypes is an effective way to achieve sustainable control of *F. verticillioides* in the field, and breeding for resistance may be accelerated by identifying genes and loci responsible for natural disease resistance. Significant advances have been made in the development of genomic tools for maize, *F. verticillioides* moulds, and their interactions over recent years. Several quantitative trait loci (QTL) and single-nucleotide polymorphism (SNP) markers for resistance to *Fusarium* deriving from QTL mapping and genome-wide association studies have been described in three different maize populations: 1. Bi-parental population; 2. Association mapping panel; 3. Multi-parent Advanced Generation Inter Crosses (MAGIC). To guide the identification of candidate genes within the identified QTL, transcriptomic and sequencing information have been exploited. Candidate genes associated with disease resistance and pathogen related-mechanisms at the *Fusarium* resistant loci have been identified on maize chromosomes 4, 5 and 7. Many of the identified candidates genes and SNPs are freely available for direct use in either molecular assisted breeding or genome editing approaches.

This work was funded by the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 678781 (MycoKey).

MAIZE BREEDING LINES AND HYBRIDS RESISTANT TO *FUSARIUM VERTICILLIOIDES* INFECTIONS

MAROCCO A., LANUBILE A., STAGNATI L.
Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.
adriano.marocco@unicatt.it

Maize is mainly affected by the fungal pathogens *Fusarium verticillioides* causing Fusarium ear rot (FER). The fungus is of concern to stakeholders as it affect crop yield and quality, contaminating maize grains with the mycotoxins fumonisins. The easiest strategy to prevent pre-harvest contamination by *F. verticillioides* is to develop maize hybrids resistant to FER, as well as to its associated mycotoxins. The objective of this investigation was to test 46 F1 hybrids, originated from different breeding programmes, for these important traits and their agronomic performances. All hybrids were planted and artificially inoculated with toxigenic strain of *F. verticillioides* at two locations in 2017, and the best performing 17 out of 46 were also tested in 2018. FER was present in all hybrids in 2017 and 2018, with percentages ranging from 6.50% to 49.50%. Seven hybrids (PC8, PC15, PC9, PC11, PC14, PC34 and PC17) presented the lowest levels of disease considering the overall locations and growing seasons, and three out of them (PC8, PC11 and PC14) were also among the less mycotoxin contaminated hybrids. The best inbred lines used in hybrid production were classified for distinctness, uniformity and stability according to UPOV guidelines. The inbreds are proposed for plant variety protection and they will be available for public and private breeding programs targeting FER.

This work was funded by the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 678781 (MycoKey).

UNDERSTANDING THE MECHANISMS OF PRE-HARVEST AFLATOXIN RESISTANCE IN PEANUT

MASSA A.N., ARIAS R.S., SOBOLEV V.S., LAMB M.C..
National Peanut ResearchLaboratory, Agricultural Research Service, United States Department of Agriculture, P.O. Box 509, Dawson, Georgia 39842, United States

Aspergillus flavus and *A. parasiticus* are opportunistic pathogens that invade peanut and other crops causing accumulation of aflatoxins. Limited understanding of the underlying mechanismsof pre-harvest aflatoxin contamination in peanut seeds has hampered the development of resistant cultivars and alternative methods of control. Our laboratory has developed a platformfor screening, characterization, and evaluation of wild *Arachis* germplasm and peanut landraces. This platform enables the rapid identification of resistance. To date, we have identified wild diploid peanut species that do not accumulate aflatoxin upon infection with aflatoxigenic *A. flavus*. This study explores chemical profiles of the defensive stilbenoids produced by peanuts in response to *Aspergillus* infection and the plant-fungus interactions thatleads to aflatoxin formation. We report gene expression changes in peanut seeds and *A. flavus* at early stage of infection.

This research was financially supported by USDA-ARS projects 6044-42000-011-00D and 6604-21000-003-00D.

ASPERGILLUS FLAVUS BIOMASS IN MAIZE AND USE OF A BIOCONTROL STRATEGY TO LIMIT AFLATOXIN PRODUCTION

MITEMA A., FETO NA. AND RAFUDEEN MS.

[1]Department of Molecular and Cell Biology, Plant Stress Laboratory 204/207, MCB Building, Upper Campus, University of Cape Town, Private bag X3, 7701, Rondebosch, Cape Town, South Africa. [2]School of Biological Sciences, University of Nairobi, P.O. Box 30596-0 0100, Nairobi, Kenya. [3]Department of Biotechnology, OMICS Research Laboratory, Vaal University of Technology, South Africa.

alfmite@yahoo.com, suhail.rafudeen@uct.ac.za and naserf@vut.ac.za

Aspergillus flavus colonization of maize can produce mycotoxins that are detrimental to both human and animal health. Screening of maize lines resistant to *A. flavus* infection together with a biocontrol strategy could help minimize subsequent aflatoxin contamination. We developed a qPCR assay to measure *A. flavus* biomass and showed that two African maize lines, GAF_4 and KDV1, had different fungal loads for the aflatoxigenic isolate (KSM014), fourteen days after infection. The qPCR assay revealed no significant variation in *A. flavus* biomass between diseased and non-diseased maize tissues for GAF_4 while KDV1 had significantly higher *A. flavus* biomass ($P < 0.05$) in infected shoots and roots compared to the control. The biocontrol strategy using an atoxigenic isolate (KSM012) against the toxigenic isolate (KSM014) showed aflatoxin production inhibition at the co-infection ratio, 50:50 for both maize lines (KDV1 >99.7% and GAF ≥ 69.4%) as confirmed by bioanalytical techniques. As far as we are aware, this is the first report in Kenya where the biomass of *A. flavus* from maize tissue was detected and quantified using a qPCR assay. Our results suggest that maize lines that have adequate resistance to *A. flavus* together with the appropriate biocontrol strategy could limit outbreaks of aflatoxicoses.

INTERACTIONS BETWEEN *ASPERGILLUS FLAVUS* AND STORED-GRAIN INSECTS IN CONVENTIONAL AND TRANSGENIC MAIZE

MANDAP J.L. [1], HELLMICH R.L.[2], MUNKVOLD G.P.[1].
[1]*Iowa State University Department of Plant Pathology & Microbiology and Seed Science Center, 2115 Osborn Dr., Ames, IA 50011, USA.* [2]*USDA-ARS Corn Insects and Crop Genetics Research Unit and Iowa State University Department of Entomology, 2333 Pammel Dr., Ames, IA 50011, USA.*
munkvold@iastate.edu

Uncontrolled grain temperature and moisture during storage can lead to insect and mold infestations that result in mycotoxin contamination. Controlling stored-grain insects can contribute to mycotoxin reduction by preventing damage that predisposes grain to fungal colonization and mycotoxin contamination. Transgenic insect resistance in maize has been shown to indirectly reduce mycotoxin contamination in the field as a result of deterred insect feeding, but this has not been demonstrated in storage. In this study, interactions between *Aspergillus flavus* and Indianmeal moth (*Plodia interpunctella*) or maize weevil (*Sitophilus zeamais*) were assessed in grain from *Bt* and non-*Bt* maize hybrids under storage conditions that are common in tropical and sub-tropical environments (32° C and 80-85% RH). Indianmeal moth larvae or maize weevil adults were infested into grain in separate jars with and without *A.flavus*. After 28 days, no insects survived in the transgenic hybrids with lepidopteran and coleopteran resistance, respectively. *A. flavus* increased mortality and reduced survivorship and growth indices of both insects. Hence, grain damage and weight loss were higher in the uninoculated grain because of greater insect feeding than in the inoculated grain. *A. flavus*-insect interactions were influenced by the presence of *Bt* proteins in the maize grain. Insect infestations increased levels of aflatoxin contamination in the non-*Bt* hybrid inoculated with 10^6 spores/ml, but did not affect aflatoxin levels in *Bt* hybrids. These results indicate that transgenes that target field pests also provide protection against storage insects and can reduce the risk of aflatoxin development in stored grain.

5-N-ALKYLRESORCINOLS AS BIOPESTICIDES: INVESTIGATING THEIR ROLE AGAINST THE ACCUMULATION OF DEOXYNIVALENOL IN DIFFERENT TRITICUM AND TRITORDEUM SPECIES

PEDRAZZANI C., VANARA F., BLANDINO M., DALL'ASTA C., RIGHETTI L.

[1]Department of Food and Drug, University of Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy. [2]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini, 2, Grugliasco, Italy.

Fusarium head blight (FHB) is a devasting fungal disease in many parts of the world and the subsequent accumulation of its mycotoxins, especially deoxynivalenol (DON) represents one of the most important threats to food safety. FHB has harmful effects on human and animal health as well as economic losses. Among the strategies developed for reducing DON accumulation in grains, the use of natural molecules is becoming increasingly important. Recently, the secondary plant metabolites alkylresorcinols (AR) have gained interest as biopesticides, because of their involvement in limiting the fungal spread in cereal crops. At this purpose, the present study aimed to investigate the correlation between saturated and unsaturated AR homologues and the accumulation of DON in different varieties of *Triticum spp*. and tritordeum, a new species not yet completely characterized. Samples were grown over two consecutive years under uniform agronomic conditions. DON and AR content was analysed using UHPLC-IMS-HRMS. Our result showed a negative correlation ($P<0.05$) between DON content and the ratio of AR homologues AR 21:0/AR 23:0, previously reported as an indicator of antifungal activity. The result obtained in the present study confirmed the involvement of AR on the accumulation of DON in wheat, finding a great diversity in the AR content in cereals depending on the genetic and environmental background.

AFLATOXIN MINIMIZATION PROGRAMME IN ROMANIA BY SELECTION AND USE OF ATOXIGENIC *ASPERGILLUS FLAVUS* AS BIOCONTROL AGENT IN MAIZE FIELD

PERRONE G.[1], SMEU I.[3], MAGISTÀ D.[1], ARCIUOLO R.[2], LOGRIECO A.F.[1], BATTILANI P.[2]

[1]National Research Council, Institute of Sciences of Food Production, Bari, Italy; [2]Università Cattolica del Sacro Cuore, Piacenza, Italy; [3]Institutul National de Cercetare-Dezvoltare pentru bioresurse alimentare, Bucharest, Romania.

antonio.logrieco@ispa.cnr.it

Recently, *Aspergillus flavus* arise as an emerging problem for maize in Southern Europe. After the first European aflatoxins outbreak in 2003, followed by severe contamination in 2012 and 2015, the prevention of aflatoxins in maize field became a relevant issue. Competitive exclusion of toxigenic *Aspergillus flavus* by atoxigenic strains is worldwide accepted as one of the most effective action to minimize aflatoxins risk. USA, Africa and Italy have developed some commercial biocontrol agent products and Eastern Europe countries start working in the same direction.
This study aimed to select an *A. flavus* biocontrol agents in Romania. Therefore, *Aspergillus* section *Flavi* strains were isolated, identified and characterized, from 139 maize flour samples, representative of the whole Romanian maize growing areas. Deletions of the aflatoxins gene cluster were investigated by multiplex PCR analyses. Microsatellite alleles were bioinformatically analysed to depict the population structure. 169 strains were confirmed as *A. flavus*; 71 strains seems to lack at least one gene of the aflatoxins cluster. Competition tests, involved 7 chemically confirmed atoxigenic strains with huge deletions in aflatoxins cluster. Microsatellites analysis identify two main group by bayesian clustering, genetic distance-based analysis and population assignment.
Strain 36.3 was selected as potential biocontrol agent, among the 7 atoxigenic strains that exhibited high aflatoxins reduction (80-96%) during in vitro competition tests. Thermally treated sorghum coated with the inoculum of this atoxigenic strain was distributed in maize field trials (approximately 1 ha) in Romania (2018 and 2019). Since, very low aflatoxins contamination was measured, even in untreated fields, during both years, the biocontrol efficacy needs to be confirmed by further field data.

The present work has received funding by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey).

to Index

THE ROLE OF PEANUT DEFENSIVE PHYTOALEXINS IN INHIBITION OF AFLATOXIN BIOSYNTHESIS IN *ASPERGILLUS* SPECIES

SOBOLEV V., ARIAS R., MASSA A., WALK T. AND LAMB M.
National Peanut Research Laboratory, Agricultural Research Service, United States Department of Agriculture, P.O. Box 509, Dawson, Georgia 39842, United States

Common soil fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, are opportunistic pathogens that invade preharvest peanut seeds. These fungi often produce carcinogenic aflatoxins that pose a threat to human and animal health through food chains and cause significant economic losses worldwide. Detection of aflatoxins and further processing of crops are mandated to ensure that contaminated agricultural products do not enter food channels. Under favorable conditions, the fungus-challenged peanut seeds produce phytoalexins, structurally related prenylated stilbenoids, capable of retarding fungal development. However, the mechanism of peanut–fungus interaction has not been sufficiently studied. The purpose of the present study was to evaluate the potential influence of peanut phytoalexins on fungal development and aflatoxin formation in the course of peanut–fungus interaction. The present research revealed that during such interaction, aflatoxin formation was completely suppressed in *A. flavus* and *A. parasiticus* strains tested, when low concentrations of spores were introduced to wounded preincubated peanuts. In most of the experiments, when fungal spore concentrations were 2 orders of magnitude higher, the spores germinated and produced aflatoxins. The research provided new knowledge on the aflatoxin/phytoalexin formation in the course of peanut–fungus interaction.

This work was financially supported by USDA-ARS projects 6044-42000-011-00D and 6604-21000-003-00D.

A MYCOTOXIGENIC PROSPECTIVE OF FUNGAL GENOME SEQUENCING: GENETIC VARIABILITY OF MYCOTOXIN CLUSTERS AT INTER- AND INTRA-SPECIFIC LEVEL

SUSCA[1] A., PROCTOR R.H.[2], VILLANI A.[1], LOGRIECO A.F.[1], HAIDUKOWSKI M.[1], AND MORETTI A[1].

[1]*Institute of Sciences of Food Production, Italian National Research Council (ISPA-CNR), Via Amendola 122/O, 70126, Bari, Italy.*

[2]*US Department of Agriculture, Agriculture Research Service, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604, USA*

Fungal genomes carry many more biosynthetic pathways than known compounds, demonstrating that the fungal kingdom has been an underexploited resource of secondary metabolites (SMs), including the mycotoxins. Knowing the diversity of biosynthetic pathways encoded in fungal genomes is therefore a powerful tool for screening their intra- and interspecific variability of mycotoxin gene clusters. Among plant pathogens, great concern is due to the occurrence of toxigenic fungi on food and feed crops, since the mycotoxin accumulation in the final products represents a serious risk for human and animal health. Among the species that produce mycotoxins *in planta*, fungi belonging to *Aspergillus* and *Fusarium* genera are the most common and show a great variability of their mycotoxin profile among species, even in close phylogenetically related species, but also at intraspecific level. We report here results of our studies conducted using Whole Genome Sequencing approach, which allow us to evaluate the: *i*) variability of Ochratoxin A (OTA) production related to the occurrence of gene (*ota*) cluster in *Aspergillus niger* clade,; *ii*) variability of beauvericin (BEA) production and BEA gene cluster occurrence in *Fusarium subglutinans* and *Fusarium temperatum*, two phylogenetic sister species where toxigenic potential is not related to real production capacity *in vitro*; *iii*) variability of trichothecenes (TRI) production in the *Fusarium equiseti/incarnatum* species complex and related variability in TRI genes cluster. *iiii*) variability of fumonisins (FBs) production and FUM genes cluster occurrence in *Fusarium proliferatum* isolated from different crops. Taken together, these data show that mycotoxin gene clusters can dramatically differ within a single species, or among very closely related species, and the lack of a given mycotoxin production, at least *in vitro* conditions, is frequently but not always related to the absence of genes cluster.

SUPPRESSING *FUSARIUM GRAMINEARUM* AND MYCOTOXINS BY APPLICATION OF MICROBIAL ANTAGONISTS ON INFECTED CROP RESIDUES.

GIMENO A.[1,2], DRAKOPOULOS D.[1], KÄGI A.[1], BÄNZIGER I.[1], LEHMANN E.[1], FORRER H.R.[1], KELLER B.[2] AND VOGELGSANG S.[1]

[1]Research Group Ecological Plant Protection in Arable Crops, Research Division Plant Protection, Agroscope, 8046 Zurich, Switzerland. [2]Molecular Plant Biology and Phytopathology, Department of Plant and Microbial Biology, University of Zurich, 8008 Zurich, Switzerland.

susanne.vogelgsang@agroscope.admin.ch

Sustainable agricultural systems require innovative and integrated methods for control of Fusarium Head Blight (FHB) in wheat to reduce the risk of mycotoxins in food and feed. Preventive actions against the dominating pathogen *Fusarium graminearum* using biological control agents on infected crop residues are a promising approach. We investigated the ability of *Clonostachys rosea* and *Trichoderma atrobrunneum* to suppress *F. graminearum* on maize residues. At first, we explored the antagonistic activity of *C. rosea* strain 016 on maize stalk pieces infected with *F. graminearum*. In contrast to other fungal candidates, *C. rosea* completely inhibited the formation of perithecia and ascospore discharge. Subsequently, the efficacy of formulations of *C. rosea* and *T. atrobrunneum* (strain ITEM908) was investigated in field experiments (2016/17, 2017/18). The collected data included *Fusarium* spore deposition during anthesis, disease symptoms as well as mycotoxin content, incidence of *Fusarium* species and *F. graminearum* DNA in harvested grains. Applications of *C. rosea* on maize stalks resulted in significantly lower FHB symptoms and reduced the deoxynivalenol (DON) content by up to 82% and 90% in the first and second year, respectively. Likewise, zearalenone (ZEN) was reduced by up to 80% in the first and by up to 90% in the second year. The efficacy of *T. atrobrunneum* was variable between years. While no significant reductions occurred in the first year, DON and ZEN were reduced by up to 80 and 90% in the second year. The great potential of *C. rosea* to reduce FHB will be further investigated in on-farm experiments.

This research was carried out in the framework of the Horizon 2020 project MycoKey "Integrated and innovative key actions for mycotoxin management in the food and feed chain" (GA 678781) and was funded by the European Union as well as the Swiss State Secretariat for Education, Research and Innovation.

MOLECULAR CLONING AND CHARACTERIZATION OF A WHEAT UDP-GLUCOSYLTRANSFERASE GENE RESPONSIBLE FOR TOLERANCE TO DEOXYNIVALENOL ACCUMULATION AND RESISTANCE TO FUSARIUM SPREAD

HE Y., ZHANG X., MA H.X., YU L.X., WU L., JIANG P.
1Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, Jiangsu, China. 2Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China.
xuzhang@jaas.ac.cn

Fusarium head blight (FHB), a devastating disease in wheat worldwide, results in yield loses and mycotoxin, such as deoxynivalenol (DON), accumulation in infected grains. DON is harmful to human and animal health, as well as facilitates the spread of FHB symptoms. The ability of a conversion of DON into DON-3-glucoside (D3G) by UDP-glycosyltransferase enzymes (UGTs) is correlated with resistance to FHB, but only few gene members in wheat have been investigated. In the present study, a Fusarium graminearum (Fg) and DON induced gene TaUGT6 in resistant variety Sumai 3 was cloned and characterized. The ORF of TaUGT6 was 2161 bp in length, encoding 490 amino acids residues. TaUGT6::GFP was subcellularly located in the plasma membrane and nuclear. The purified TaUGT6 protein was able to convert DON into D3G in vitro. Transformation of TaUGT6 into Arabidopsis conferred enhanced DON tolerance when grown on agar plates containing 30 ppm of DON. Furthermore, over expressing TaUGT6 in wheat showed significant better resistance to Fusarium spread at 9 to 15 days after Fg inoculation. The promoter deletion analysis showed that sequences between 431 and 1200 bp upstream of the transcriptional start site were required for DON-induced expression. Overall, this study gave a useful insight into a novel UGT gene for FHB resistance in wheat.

This work was partially supported by the National Key Project for the Research and Development of China (2016YFD0101802, 2017YFE0126700), National Natural Science Foundation of China (31561143004, 31801727), and European Union Horizon 2020 Mycokey project (EU678781).

to Index

Session 4

Food and feed Remediation, Intervention & Processing

THE INFLUENCE OF PROCESSING PARAMETERS ON THE MITIGATION OF DEOXYNIVALENOL DURING INDUSTRIAL BAKING

SUMAN M.[1], STADLER D.[2], BERTHILLER F.[2], LAMBERTINI F.[1], BUESCHL C.[2], WOELFLINGSEDER L.[3], HAMETNER C.[4], SCHWARTZ-ZIMMERMANN H.E.[2], HELLINGER R.[2], SULYOK M.[2], LEMMENS M.[2], MARKO D.[3], SCHUHMACHER R.[2], KRSKA R.[2]

[1]Barilla G. R. F.lli SpA, Advanced Laboratory Research, Parma, Italy; [2]Department IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria; [3]Department of Food Chemistry and Toxicology, University of Vienna, Austria; [4]Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria

michele.suman@barilla.com

Deoxynivalenol (DON) is the most prevalent mycotoxin in cereal commodities. Although the population of industrial nations is exposed to DON mainly due to the consumption of bread and other bakery wares, it is not clear whether the toxicological impact of DON can be mitigated during industrial baking. After 30 years of research, the knowledge of degradation products that are formed from DON during baking and their toxicity is still incomplete. Furthermore, the extent of possible DON reduction is highly controversial. Although most studies found a reduction of DON, some even up to 50 %, increases of up to 40 % were also reported. To determine whether the toxicological impact of flour that is contaminated with DON can be mitigated during the industrial production of bakery wares we:

- elucidated the full spectrum of DON degradation products by an untargeted liquid chromatography high resolution mass spectrometry approach using ^{13}C labelling,
- compared the cytotoxicity of an important degradation product to DON,
- determined the amount of DON that is degraded during the production of crackers, biscuits and bread by targeted LC-tandem mass spectrometry (MS/MS) analysis,
- evaluated the influence of different process parameter (*e.g.* baking temperature and time) on DON mitigation.

Our holistic approach in combination with the currently most accurate analytical methodology to determine DON degradation enabled us to clarify i) how much DON is degraded during the production of crackers, biscuits and bread, ii) whether DON degradation actually causes less toxicity and iii) how the choice of process parameter can maximize DON mitigation.

Toxins **2019**, 11, 317; doi:10.3390/toxins11060317

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012 for the MyToolBox project.

BIOMIN CONTRIBUTION TO MYCOTOXIN RISK MITIGATION.

MUELLER A.*, MARCHIORO A., HOFSTETTER U.
BIOMIN Holding GmbH, Getzersdorf, Austria.
anneliese.mueller@biomin.net

Contamination of feed with mycotoxins is of global concern and is negatively affecting animal health and performance and with this feed and livestock industry. ERBER Group (BIOMIN, ROMER LABS and others), is committed to natural solutions to support feed and food safety. These solutions include methods to rapidly analyze mycotoxins, as well as highly sensitive and accurate methods to analyze many different and so called "emerging" mycotoxins, offered by ROMER LABS. Data received from mostly routine customer feed analysis, is combined in the annual Mycotoxin Survey by BIOMIN. This Survey offers information about contamination levels in different raw commodities and finished feed. Results from 2019 are based on 21287 samples from 86 countries and show again high prevalence of *Fusarium* toxins. Worldwide, fumonisins (FUM) were detected in 70% of samples with an average of positives of 1800 ppb (median 730 ppb), followed by deoxynivalenol (DON) (68%, average 699 ppb, median 350 ppb) and zearalenone (ZEN) (52%, average 138 ppb, median 48 ppb). Europe is mostly affected by DON, which showed high prevalence in corn (83%, average 726 ppb, median 355 ppb) and cereals (56%, average 1057 ppb, median 343 ppb). Several approaches exist to decontaminate feed (chemical and physical), but are time consuming and cannot fully remove mycotoxins. BIOMIN offers feed additives that are detoxifying various mycotoxins in the gastrointestinal tract of animals backed by EU authorizations. BIOMIN's solution is based on adsorption, biotransformation as well as bioprotection supporting animals` health.

PULSED ELECTRIC FIELD (PEF) AS AN INNOVATIVE TOOL FOR DECREASING *FUSARIUM* MYCOTOXINS IN THE BARLEY -MALT PRODUCTION CHAIN?

HAJSLOVA J., STRANSKA M., PRUSOVA N., BEHNER A.
University of Chemistry and Technology, 166 28 Prague, Czech Republic;
jana.hajslova@vscht.cz, milena.stranska@vscht.cz

Effective control of *Fusarium* infection of barley grains is of a high concern for malting industry due to the risk of mycotoxins production. To improve safety of the final products (malt and beer), introduction of approaches reducing moulds growth during malting is highly desirable. Although number of physical, chemical and biological methods has been tested, none of them has met all the required criteria regarding effectiveness, and quality of malt used for brewing. In this context, pulsed electric field (PEF) represents a challenging technological intervention. This non-thermal technique has been introduced for microbial inactivation with an objective to maintain the nutritional properties and physic-chemical characteristics of respective products. In this study we treated by PEF Bojos malting barley artificially infected in field by *F. culmorum, F. graminearum, F. sporotrichioides, F. poae*. Using ultra-high performance liquid chromatography coupled with tandem high resolution mass spectrometry (U-HPLC-HRMS/MS, Q-orbitrap mass analyser), the presence of a wide range of mycotoxins including NIV, DON, D3G, ADONs, NEO, HT2, T2, Enns, BEA and ZEA, was detected in barley samples. Depending on PEF parameters setting, the decrease of most of targeted mycotoxins, up to 40%, occurred. To explain these observations, possible structures of mycotoxins transformation products were predicted by Zeneth software (Lhasa Limited). From 2289 structures (including isomers), several oxidized forms of trichothecenes together with products of oxidative degradation of enniatines and beauvericine, were confirmed. The U-HPLC-HRMS/MS based metabolomic fingerprinting followed by multivariate analysis of generated data enabled identification of lysophospholipids as characteristic markers of PEF treatment. These compounds, known to be connected to stress signalling pathways in plants, might indicate an increased stress level in the PEF treated barley grains.

AFLATOXIN M_1 IN CHEESE: STATE OF THE ART IN ENRICHMENT FACTORS DETERMINATION

PECORELLI I.[1], BRANCIARI R.[2], BIBI R.[1], ROILA R.[2],
VERDINI E.[1], VALIANI A.[1]

[1]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Via Salvemini 1, 06126-Perugia, Italy; [2]Department ofVeterinary Medicine, University of Perugia, Via San Costanzo 4, 06126-Perugia, Italy

i.pecorelli@izsum.it

AFM_1 contamination of cheese, derives indirectly from milk contamination. As AFM_1 is bound to the milk casein fraction, the cheese obtained results at higher AFM_1 concentration than milk. Several studies have been published regarding the fate of AFM_1 during cheese production, and the evaluation of enrichment factor (EF) was defined as the ratio among concentration of AFM_1 in cheese and its concentration in milk. Nevertheless specific maximum levels for AFM_1 in dairy products, such ascheese, are still lacking. According to Article 2 of (EC) Regulation No. 1881/2006, the EF is an important parameter that has to be established to evaluate the maximum level of contaminants in dried, diluted, processed, and composed foodstuffs aiming to ensure that cheese has been produced from compliant milk. In 2013 Italian Ministry of Health[1] recommended the adoption of two provisional EFs equals to 3.0 and 5.5 for soft and hard cheeses respectively. Many experimental studies were published by the authors to determine and evaluate EFs in semi-hard ewe's and cow's cheeses[2,3,4] concluding that EF is correlated to the cheese yield and composition of raw material has to be taken into account as well. Afterwards Ministry of Health has set up a working group of experts to better define EF in different cheese categories. Regarding cow's milk cheese, working group has identified, using MFFB (Moisture content on a Free Fat Basis), four categories of cheese and, consequently, four new EF were adopted. For different origin cheeses data are still limited and further investigations are necessary.

References

1. Italian Ministry of Healt: Parere del Comitato Nazionale della Sicurezza Alimentare n.13 del 10/06/2013.
2. Pecorelli, I., R. Branciari, R. Ortenzi, M. Ciriaci, S. Checcarelli, R. Roila, A. Capotorti, G. Spaccini, and A. Valiani. 2018. Evaluation of the concentration factor of aflatoxin M_1 in a semi-hard Pecorino cheese obtained from naturally contaminated milk. Food Control 85: 194–198.
3. I. Pecorelli, R. Branciari, R. Roila, R. Bibi, D. Ranucci, A. Onofri, and A. Valiani. Evaluation of Aflatoxin M_1 Enrichment Factor in Semihard Cow's Milk Cheese and Correlation with Cheese Yield. Journal of Food Protection: July 2019, Vol. 82, No. 7, pp. 1176-1182.
4. Ivan Pecorelli, Raffaella Branciari, Rossana Roila, David Ranucci, Rita Bibi, Andrea Valiani. Evaluation of Aflatoxin M_1 Enrichment Factor in different Cow Milk Cheese hardness category. Italian Journal of FoodSafety (accepted) 2020.

BIO-PRESERVATION ACTIVITY OF FERMENTED WHEY BY LACTIC ACID BACTERIA IN LOAF BREAD

LUZ C., D'OPAZO V., LLUENCA F., VITALI M.,
CALPE J., MAÑES J., MECA G.*

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicente Andrés Estellés s/n, 46100-Burjasot, Spain.

giuseppe.meca@uv.es

Whey is a waste product from cheese industry. However, it contains proteins that have a high nutritional value and are an important source of bioactive compounds. Food deterioration caused by toxigenic fungi is one of the challenges of food safety. The antifungal activity of whey milk fermented by lactic acid bacteria (LAB) and the use of this ingredient as novel application of bio-preservation of loaf bread and as reduction mycotoxins method in food were performed. Whey was fermented by nine selected LAB for 72 h at 37 ºC. and the antifungal activity against 28 strains of toxigenic fungi were determined. Subsequently, the FW was incorporated into the bread formulation, and the pH; antimicrobial metabolites, such as organic acids and volatile organic compounds (VOCs); total phenolic content; and DPPH radical-scavenging activity of FW and breads were characterised. A study of shelf life of breads inoculated with a suspension of *Penicillium verrucosum* (ochratoxin A producer) and by natural contamination was carried out to study the reduction of fungal growth and mycotoxin production compared to bread without additive, bread with additive calcium propionate and bread with whey non-fermented. FW showed MIC and MFC values in range of 8-250 g/L. Breads in which 100% of the water was replaced with FW by *L. plantarum* TR7 evidenced an improvement in the shelf-life of 4-days compared with the control with calcium propionate 0.3%. Bread with FW evidenced a reduction of fungal growth of 0.5-1 log spores/g and an ochratoxin A production in range of 85-100%.

This research study was supported by the Ministry of Science and Innovation (PID2019-108070RB-100) and by the project Prometeo/2018/ 126 supported by Generalitat Valenciana.

THE CIRCULAR ECONOMY SOLUTION TO AFLATOXIN: TRANSFORMING TOXIC CROPS INTO HEALTHY FOOD AND FEED

ALINOVI L.
luca@aflazero.com

Aflazero was incorporated in 2017 to commercialize the first viable technology to remove aflatoxin from grains, nuts and pulses. This technology is based on 10 years of research and development, a portion of which was funded by the European Union. The initial success in the lab (removing over 95% of aflatoxin from contaminated grains) has now been scaled up for country-level applications. Aflazero has assembled a highly experienced team to apply this technology to core problems faced by governments (food security and health) and commodity traders and food processing companies (exporting aflatoxin-contaminated commodities to Europe and US markets).

Kenya has been very active in addressing mycotoxin contamination, including incinerating its strategic grain reserve and banning companies from the market for selling unsafe products (such as milk, flour and peanut butter). Kenyan authorities are working with Aflazero to transform seized contaminated maize into healthy animal feed, which otherwise would have been destroyed. The first such treatment plant will be established in Kenya with the support of International Finance Corporation (IFC) and National Cereals and Produce Board (NCPB), as well as other key partners including the Kenya Ministry of Agriculture, Ministry of Health, World Bank and UNIDO.
Following the successful implementation of the pilot plant, our intention is to rapidly scale up production of the commercial plants and begin decontaminating Kenya's entire strategic grain reserve. At the same time, we will work with research and commercial partners to identify further strategic crops to target for decontamination and export.

EFFECT OF MILLING PROCESSES ON MONILIFORMIN DISTRIBUTION IN WHEAT AND MAIZE FRACTIONS IN COMPARISON TO THE TARGET MYCOTOXINS DEOXYNIVALENOL AND FUMONISINS

SCARPINO V., VANARA F., BLANDINO M.*

Università degli Studi di Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

massimo.blandino@unito.it

The effect of milling processes on moniliformin (MON) distribution in wheat and maize milling fractions, in comparison to the target mycotoxins deoxynivalenol (DON) and fumonisins (FBs), was evaluated in 1 wheat and 3 maize commercial lots, subjected to roller-milling technology. The sampled fractions of each process were collected according to European Commission Regulation (EC) No 401/2006.

Through maize dry-milling, in comparison to the pre-cleaned whole grain:

- the cleaning step on average reduced the FBs, MON and DON_{TOT} (sum of DON, deoxynivalenol-3-glucoside, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol) contents by -47%, -45% and -35%, respectively.
- the animal feed flour increased the FBs, MON and DON_{TOT} contents, by 3.0, 1.6 and 2.1 times, respectively.
- the germ presented reduced FBs (-58%) and MON (about -40%) contents, while DON_{TOT}, increased by more than 2.5 times.
- within endosperm products (maize flour, break meal and pearl meal), the maize flour (fraction with the lowest particle size) showed the smallest reduction (-24% for FBs, -41% for MON and -61% for DON_{TOT}), underlining an inverse relationship with the particle size.

Through wheat milling, DON_{TOT} and MON were reduced respectively by -38% and -15% in refined flour, and by -41% and -33% in germ, while increased their content in bran (+177% and 65%), shorts (+113% and +77%) and middlings (+94% and +18%).

The weaker decontamination of MON in maize and wheat milling fractions and the increase of DON_{TOT} in the maize germ points to a higher risk of exposure for the end consumers.

This research was financially supported by the Project ALIMAIS and WHITEGRITS (Regione Piemonte, Rural Development Programme FEARS. 2007-2013) and ACRYSAFE Regione Piemonte (POR FESR 2014–2020).

PROTEOMIC CHANGES AFTER FERMENTED WHEY AND PUMPKIN EXPOSURE AGAINST MYCOTOXINS TOXICITY ON LYMPHOBLASTOID CELL LINE

CIMBALO A.[1]*, FRANGIAMONE M.[1], LOZANO M.[1], VILA-DONAT P.[1], MANYES L.[1]

[1]*Laboratory of Food Chemistry and Toxicology. Faculty of Pharmacy. Universitat de València. Carrer Vicent Andrés Estellés s/n. 46100 Bujassot. Spain.*

alscim@uv.es

Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are natural carcinogenic toxins which contaminate crops and a wide variety of food materials. This work combined the beneficial effect of pumpkin and fermented whey (WF) against AFB1 and OTA cytotoxicity in Jurkat cells, by focusing on its proteomics profile. To carry out the study, cells were exposed during 7 days to: a) AFB1 and OTA (100 nM each) dissolved in DMSO solvent, individually and in mixture; b) to an intestinal digest of bread prepared with 20% of lyophilized pumpkin (PID) and mycotoxins (AFB1 and OTA); c) to an intestinal digest of bread prepared with the same conditions but enriched with 20% pumpkin and WF (PID+WF). Proteins were extracted from cell cultures and subsequently digested with trypsin to be further analyzed by liquid chromatography coupled with quadrupole time of flight system (Q-TOF). A total of 496 unique proteins at 1% false discovery rate was detected for DMSO conditions, 352 for PID and 316 for PID+WF using Spectrum Mill software (Agilent). Afterwards, the differentially expressed proteins have been statistically evaluated and filtered by abundance through Mass Professional Profiler software (Agilent) ($p<0.05$). DAVID database allowed the identification of proteins involved in gluconeogenesis, antioxidant activity and nucleosome assembly. Among these proteins, the expression of protein cyclin A2, which is involved in limiting carcinogenic cells growth, was lower in presence of both functional ingredients. Similarly, after PID+WF treatment, histones' H2A, H2B, H2C, H3 and H4 expression was increased. Based on these findings, functional ingredients can act as protectors against genomic stress caused by mycotoxins, preventing the loss of vital cell functions and paralyzing the growth of carcinogenic cells.

This work was supported by the Spanish Ministry of Science and Innovation (PID2019-108070RB-I00-ALI) and the Generalitat Valenciana (PROMETEO/2018/126).

ANTIFUNGAL EFFICACY OF FOOD INDUSTRIES SANITIZERS AGAINST TOXIGENIC FUNGI

LEMOS J.G.[1], STEFANELLO A.[1], GARCIA M.V.[1], MAGRINI L.N[1], SILVA M.[1], COPETTI M.V.[1].

[1]*Federal University of Santa Maria, Center of Rural Sciences, Department of Technology and Food Science, 1000 Roraima Avenue, 97105-900, Santa Maria, RS, Brazil*

mvc@smail.ufsm.br

The presence of mycotoxin-producing fungi in food products represents a public health concern worldwide, since them can produce toxic compounds during some product maturation or storage. Among the main strategy adopted by the food industries to eliminate these fungi from their manufacturing environment is sanitation, preventing food contamination. This study aimed to test the efficacy of the most used agents for industrial sanitation, in three concentrations each: benzalkonium chloride (BC) 0.3%, 1.2%, 2%; iodine(IO) 0.2%, 0.6%, 1%; peracetic acid (PA) 0.3%, 0.6%, 1%; and sodium hypochlorite (SH) 0.5%, 0.75%, 1% against strains of aflatoxigenic (*Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius*), and ochratoxigenic (*Aspergillus carbonarius, Aspergillus ochraceus, Aspergillus niger, Aspergillus westerdijkiae*) Aspergilli, comparing with the standard strains *Aspergillus brasiliensis* ATCC16404 and *Candida albicans* ATCC2443, using the methodology proposed by the European Committee for Standardization (CEN). According to CEN to be considered effective, the sanitizer must reduce at least 3log of the initial population of a microorganism. In general, IO is the best sanitizer for controlling both afla and ochratoxigenic Aspergilli, followed by BC, however the higher efficacy of BC was against aflatoxigenic strains. PA was the most effective agent against the ochratoxigenic yellow Aspergilli and *A. carbonarius,* but the other black Aspergilli, including the standard strain, were resistant to PA in all tested concentrations but sensitive to BC in the highest concentration. Even extensively used for food industries sanitation, according to our results SH should not be used for the control of toxigenic Aspergilli due its inefficacy.

This study was supported through grants by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) -Finance Code 001, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) -Process 309691/2015-0.

SENSITIVITY OF BAKERY SPOILAGE FUNGI TO THE MAIN BREAD PRESERVATIVES AT DIFFERENT PH

MORO C.B.[1]; LEMOS J.G.[1], STEFANELLO A.[1]; GARCIA M.V.[1]; BERNARDI A.O.[1]; COPETTI M.V.[1*].

[1]*Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-graduação em Ciência e Tecnologia dos Alimentos. Av. Roraima, 1000, CEP 97105-900, Santa Maria, RS, Brazil.*

mvc@smail.ufsm.br.

Organic acids are usually the first choice preservatives to control fungal spoilage and extend the shelf life of baked goods, but their effectiveness is pH dependent and fungal resistance has been reported. This study evaluated the inhibitory capacity of acetic, sorbic and propionic acids against the main spoilage fungi of bakery products at different pH values through macrodilution. For determination of the minimum inhibitory concentration (MIC), these preservatives were confronted with *Penicillium roqueforti, Penicillium paneum, Aspergillus pseudoglaucus, Aspergillus montevidensis* and *Hyphopichia burtonii* strains isolated from spoiled bakery products. The concentrations tested were: (i) sorbic acid 0, 1, 2, 4, 8, 16 and 32 mM; (ii) propionic acid 0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1,024 mM and (iii) acetic acid 0, 25, 50, 100, 200, 400, 600 and 800 mM; then adjusting the pH to 4.5; 5.0; 5.5 and 6.0 to simulate buffering induced by food components. Sorbic acid was the most effective against all species evaluated, followed by propionic and acetic acids. The MICs of each preservative usually doubled with every 0.5 pH increase. *H. burtonii* strains were the most sensitive to sorbic acid whereas *P. roqueforti* were the least. Both *P. roqueforti* and *P. paneum* were the most resistant to propionic acid, requiring twice the amount of this preservative for inhibition when compared to the *A. pseudoglaucus, A. montevidensis* and *H. burtonii* tested strains. *P. roqueforti* and *P. paneum* also showed the highest MICs to acetic acid. Propionic acid concentrations usually allowed in baked goods are lower than the concentrations required to inhibit the most resistant isolates tested. The same is true for sorbic acid at the highest pH values.

This study was supported through grants by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) -Finance Code 001, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) -Process 309691/2015-0.

ANTIFUNGAL AND ANTIMYCOTOXIGENIC ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM WHEY.

ESCRIVÁ L.[1], AGAHI F.[1], LUZ C.[1], CALPE J.[1], MANYES L.[1], FONT G.[1], MAÑES J.[1], MECA G.[1], VILA-DONAT P.[1]

[1]*Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.*

Biological detoxification of mould food spoilage and mycotoxin contamination by lactic acid bacteria (LAB) exhibits high potential on a cost-effective and large scale. The aim of the present study was to evaluate the antifungal and antimycotoxigenic activity of several LAB isolated from milk whey. The antifungal activity of twelve LAB (B1, B2, B3, B4, B5, B6, B7, B9, B10, BS4, BS6, BS7) against fungal strains from *Penicillium (P. expansum, P. digitatum, P. commune), Aspergillus (A. flavus, A. carbonarius, A. niger)* and *Fusarium (F. proliferatum, F. graminearum)* was evaluated by the agar diffusion test in PDA. Fermented milk whey with the studied LAB, lyophilized and concentrated (400g/L) was inoculated (n=3) with toxigenic fungi (100µl, $5x10^4$ spores/ml) and incubated 72h (25°C). LAB antimycotoxigenic effect against OTA (500µg/L) and AFB1 (100µg/L) was evaluated in MRS (37ºC) by HPLC-MS/qTOF analysis after incubation at different time points (0, 2, 4, 6, 24, 48, 72h). All toxigenic fungi showed growth reduction by at least one LAB, with P. *commune and F. proliferatum* as the most sensitive species both reduced by six LAB; followed by *A. niger and F. graminearum*. B4 was the most effective LAB active against all strains but one, followed by BS7, B3, B4, BS4 and BS6. MIC and MFC values were 12.5-100 g/L and between 50 and >200 g/L, respectively. Significant mycotoxin reductions ($p<0.05$) were observed increasing over the time. Nine LAB reduced OTA (12-40%) with B3, B10, and BS7 as the most active ones. Five LAB reduced AFB1 (11-35%), highlighting B3 activity.

This research was financially supported by the Project PID2019-108070RB-100 (Ministry of Science and Innovation, Spain) and PROMETEO/2018/126 (Generalitat Valenciana, Spain).

PUMPKIN EXTRACT AND FERMENTED WHEY ALLEVIATED AFB1 AND OTA-INDUCED ALTERATION ON NEURONAL DIFFERENTIATION *IN VITRO*

FRANGIAMONE M.[1], ALONSO-GARRIDO M.[1], CARFAGNINI C.[1], FONT G.[1], MANYES L.[1].

[1]*Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, Universitat de València, Burjassot, Spain.*

Due to the globalization and long-term grain storage, mycotoxins have become a great issue in food safety being the main contaminants of foodstuffs. In particular, AFB1 and OTA are the most toxic and studied. Although several articles suggested AFB1 and OTA-ability to cross the blood brain barrier and disrupt its integrity, the effects on brain are not well reported. Therefore, the aim of this study was to evaluate through a microscopic, flow cytometric and transcriptomic approach the effect of AFB1 and OTA on neuronal differentiation. Moreover, the possible protective role of carotenoids and fermented whey, functional ingredients selected for their antioxidant and antifungal proprieties, was evaluated. SH-SY5Y-cells ongoing differentiation were exposed during 7 days to bread extracts (diluted 1:50 in medium), obtained from a simulated human digestion *in vitro*. Bread contained pumpkin and fermented whey (both at 1% w/w), individually and in combination, along with mycotoxins (AFB1:60 nM, OTA: 240 nM, AFB1+OTA: 60+240 nM), which doses were normalized after digestion in 0.1% of methanol. This solvent concentration was also used as control. As regards immunofluorescence results, OTA and mixture exposure impaired in a greater extent the expression of neuronal markers analyzed, bIII-Tubulin and Dopamine, suggesting their negative effect on neuronal differentiation. However, these outcomes were reversed upon pumpkin extract and fermented whey administration, confirming their protective role at neuronal level. Regarding flow cytometry analysis, any remarkable differences were found. Finally, these findings are being confirmed through a RT-qPCR analysis, evaluating the expression of neuronal differentiation and cell cycle related genes.

This research was supported by Spanish Ministry of Science and Innovation Project (PID2019-108070RB-I00- ALI) and PhD grant (BES-2017-081328).

ENZYMATIC TRANSFORMATION OF AFLATOXIN B_1 BY RH_DYPB PEROXIDASE AND CHARACTERIZATION OF THE REACTION PRODUCTS.

LOI M.[1], RENAUD J.B.[2], ROSINI E.[3], POLLEGIONI L.[3], VIGNALI E.[3], HAIDUKOWSKI M.[1], SUMARAH M.W.[2], LOGRIECO A.F.[1], MULÈ G.[*1]

[1]Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR), Via G. Amendola 122/O, 70126 Bari, Italy; [2]London Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street London, Ontario, Canada, N5V4T3; [3]Department of Biotechnology and Life Sciences, University of Insubria, via Dunant 3, 21100 Varese, Italy.

giuseppina.mule@ispa.cnr.it

Aflatoxins (AFs) are fungal secondary metabolites which contaminate several staple food and feed commodities worldwide. Aflatoxin B_1 (AFB_1) is highly toxic and hepatocarcinogenic in humans. AF's exposure, especially in food-insecure countries, increases the rate of liver cancer, child mortality, malnutrition, immune suppression and hepatotoxicity, also in livestock animals.

Biological methods, such as enzymatic biotransformation, represent a mild and environmental friendly method to reduce AFs contamination. Different peroxidases have been shown to degrade several mycotoxins, including aflatoxin B_1 (AFB_1). Therefore, the aim of this study was to investigate the degrading capability of a recombinant type B dye decolorizing peroxidase (Rh_DypB) towards AFB_1. *In vitro* assays were set up in 50 mM sodium malonate buffer, pH 6.0 containing 2 mM $MnCl_2$ and AFB_1 (1 µg/mL) using different enzyme and H_2O_2 concentrations. Kinetic parameters on AFB_1 were assessed, its reduction was assessed by HPLC during a 96 hours bioconversion time and the reaction products were characterized by mass spectrometry analysis.

AFB_1 was reduced up to 96% using low enzyme and H_2O_2 dosages; an approximative maximal rate of 0.0021 µg/mL x min and a K_m of 3.2 mM were estimated at the optimal enzyme and H_2O_2 concentrations. AFB_1 was quantitatively converted to the hydroxylated metabolite AFQ_1, which is known to possess lower acute toxicity and mutagenicity than AFB_1.

Further studies are currently ongoing to investigate the mechanism of action of Rh_DypB through the use of enzyme mutants and to identify other reaction products other than AFQ_1.

Rh_DypB application could represent an effective method to reduce AFs contamination, especially in highly contaminated feed commodities.

This research was financially supported by H2020-E.U.3.2-678781- MycoKey-Integrated and innovative key actions for mycotoxin management in the food and feed chain.

ANTIFUNGAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM CITRUS FRUITS AND METABOLOMIC PROFILE OF CITRUS CONTAMINATING MYCOTOXIGENIC FUNGI.

C. LUZ[1], C. STRACQUADANIO[1], T. NAZARETH[1], R. TORRIJOS[1], J. MAÑES[1], G. MECA[1].

[1]*Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicente Andrés Estellés s/n, 46100-Burjasot, Spain.*

carlos.luz@uv.es

Fungal rots are the leading cause of postharvest losses (about 30%) of citrus and can greatly reduce its shelf life. Some postharvest fungal pathogens of citrus fruits produce mycotoxins that can pass through and be found in juices.

The aims of this study were to isolated bacteria from citrus fruits, explored their antagonistic properties against the main mycotoxigenic pathogens of citrus fruits, belonging to *Aspergillus* spp., *Penicillium* sp., *Fusarium oxysporum* and *Alternaria alternata*.

Isolated bacteria were tested against the pathogens with overlay assay that showed inhibition by direct contact. Antifungal bacteria were identified by MALDI-TOF/MS and fermented in MRS broth to produce secondary metabolites with antifungal activity. Preliminarily, the 13 cell-free supernatants (CFS) were evaluated by agar diffusion assay in which the activity was established by the presence of the inhibition halo. Subsequently, the minimum inhibition concentration and the minimum fungicidal concentration (MIC and MFC) were determined. In addition, antifungal compounds such as organic acids, phenolic acids, and volatile organic compounds (VOCs) present in CFS were identified by ESI-LC-MS-TOF and GC-MS. Also, metabolomic profile of citrus contaminating fungi was determined by ESI-LC-MS-TOF. A total of 30 strains were isolated of which 13 isolates, identified belonging to the genera *Pediococcus*, *Lactobacillus* and *Leuconostoc*, showed clear zones of inhibition on the tested pathogens. The cell-free supernatants (CFS) of 13 isolates inhibited the growth of most tested pathogens, in particular the CFS of isolates 5H1, 5L1 and N2B2 showed good activity with MIC values between 15.6-125 mg/mL and MFC values between 15.6-250 mg/mL.

This research study was supported by the Ministry of Science and Innovation (PID2019-108070RB-100) and by the project Prometeo/2018/ 126 supported by Generalitat Valenciana.

to Index

EFFICACY OF FOOD GRADE PRESERVATIVES FOR THE CONTROL OF *ASPERGILLUS FLAVUS* POPULATIONS AND AFLATOXIN B_1 CONTAMINATION IN RED CHILLIES AND CHILLI POWDER

AL-JAZA, D.[1,2], MEDINA, A.[1], MAGAN, N.[1]

[1]*Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, Beds. MK43 oAL, U.K.,* [2]*Analyses & Pathology, Science College, Thi-qar University, Iraq.*

Chillies are an important spice in many regions of the world. During the production and processing phases they are prone to infection by mycotoxigenic fungi, especially *Aspergillus* Section *Flavi* species and contamination with aflatoxins. There is significant interest in controlling aflatoxin B_1 (AFB_1) contamination in such spices to ensure they remain below the legislative limits for human consumption. The objective of this study was to examine potential efficacy of food grade preservatives for the control of A. flavus populations and AFB1 contamination relative to the legislative limits. A number of salts of aliphatic acids, anti-oxidants and slow release preservatives were screened for efficacy against A. flavus in vitro and in situ on chilli-based medium and on whole red chillies. The ED_{50} and ED_{90} concentrations showed that sodium metabisulphite (NaMBS) was particularly effective. Detailed studies in stored chilli powder and whole red chillies were carried out when stored at 30°C for up to 20 days under different water availability conditions (0.70, 0.80, 0.90, 0.95 a_w). *In situ* studies with chilli powder and whole red dry chillies (naturally contaminated or + conidial inoculum of *A. flavus*) showed that at 0.70 and 0.80 a_w, NaMBS treatments of 1000 and 2000 mg/L reduced AFB_1 contamination levels to below the legislative limits for spices. However, at 0.90 and 0.95 a_w, AFB_1 contamination of stored chilli powder, with or without additional *A. flavus* inoculum, resulted in contamination levels above the legislative limits, even with 2000 ppm NaMBS after 20 days storage at 30°C. *A. flavus* populations were reduced by 1000-2000 mg/L of the preservative during storage of whole red chillies at 30°C for up to 20 days. However, effective AFB_1 control was only achieved with 2000 mg/L NaMBS treatment after both 10 and 20 days storage. Studies with commercial laminated sheets containing immobilised NaMBS with slow release of SO_2 properties significantly reduced fungal populations and effectively controlled AFB_1 contamination of the stored and packaged whole red chillies. The practical use of such approaches for reducing the risks of AFB_1 in spices is discussed.

POTENTIAL APPLICATION OF LACTIC ACID BACTERIA FOR THE FRUITS BIOCONTROL AGAINST MYCOTOXYGENIC FUNGI

D´OPAZO V.[1], LUZ C.[1], TORRIJOS R.[1], NAZARET T.[1], QUILES J.[1], MAÑES J.[1], MECA G.[1]

[1]*Departemento de Medicina Preventiva, Facultat de Farmacia, Universitat de Valencia, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain.*

Microbial fermentations have long represented a way of natural biopreservation of raw materials. Lactic acid bacteria (LAB) are QPS (qualified presumption of safety) and fermentative microorganisms therefore a good alternative to chemicals to be applied in food preservation. A total of 9 LAB isolates from tomato and sourdough bread were screened for antimicrobial activities against toxigenic fungi belonging to the genus *Fusarium*, *Penicillium*, *Alternaria*, and *Aspergillus* using agar diffusion test, overlay method, and Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) determination. In addition, a characterization and identification of main volatile organic compounds (VOC) from MRS fermented by each bacteria during 72h was carried by CG-MS. Also, the antifungal activity of the most active LAB was tested by spraying fermented MRS on a 250 mg/mL concentration at injured tomatoes previously inoculated with *Aspergillus flavus* and *Penicillum expansum.* Fermented mediums showed MIC ranged from 1.6-100 mg/mL and MFC from 3.1-100 mg/mL. Genus *Aspergillus* showed the highest resistance, while *Fusarium* was the most sensitive. Principal detected VOC's were pyrazines, from 39 to 75 %. The treatments in tomatoes inoculated with *A. flavus* was not effective. Nevertheless, a significative growth reduction of fungi was achieved in the tomatoes inoculated with *P. expansum*, microbial count was reduced by 1.98 to 3.89 $\log_{10}$ spores per gram of tomato in compared to the treated with non- fermented MRS. Future assays will be performed on other fruits affected by *Penicillium* infections.

This work was supported by the MYCOKEY project, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 678781.

OPTIMIZATION OF STORAGE PARAMETERS FOR AFLATOXIN PRODUCTION IN ASPERGILLUS PARASITICUS INOCULATED WHEAT FLOUR USING RESPONSE SURFACE METHODOLOGY

AKINOLA S.A., ATEBA C.N.,MWANZA M.*
Center for Animal Health Studies, Faculty of Natural and Agricultural Sciences, North West University, Private Bag X2046, Mmabatho 2745, South Africa
Mulunda.mwanza@nwu.ac.za

The storage parameters for aflatoxin production in *Aspergillus parasiticus* inoculated wheat flour was optimized through the response surface methodology. Twenty experimental runs were developed composing of independent variables (incubation temperature (A), time (B) and (C) moisture content) and responses (aflatoxins concentrations; AFB1, AFB2, AFG1, AFG2 and AFTOT). A central composite face-centered design was used with lower and upper limits respectively; A (25 - 35 ^{o}C), B (7 - 15 days) and C (15 -25%) while the un-inoculated wheat flour served as negative control. Aflatoxin production was determined using High Performance Liquid Chromatography (HPLC) according to standard procedures. Numerical and graphical process variables optimisation were done, adequate models were predicted and optimal point prediction for aflatoxin concentration was determined. AFG1 concentrations ranged from 0 to 360.06 µg/g, AFG2 (0 - 446.94 µg/g), AFB2 (0 - 488.77 µg/g), AFB1 (0 -20666.6 µg/g) and AFTOT (17.09 -21851.09 µg/g). Aflatoxin concentration increased with increase in "B" and "A" but decreased with prolong "B". AFB1 concentrations in *Aspergillus parasiticus* inoculated wheat flour increased at prolonged "B" and "A" at constant "C" (12.09%). A reduced cubic model was significantly adequate to describe the relationship between process variables and responses (AFG1 and AFG2), cubic model (AFB1 and AFTOT) and a transformed square root cubic model for AFG2 concentrations ($p \leq 0.05$). "A" influenced AFG1 production than "C" while "C" and "A" had no significant effect on AFG2 production. Process variables "AB" influenced AFB2 concentrations than "C" while "A" had a significant effect on the AFTOT production than "B" ($p \leq 0.05$). The predicted (R^2) and adjusted coefficient of regression (adj R^2) were in reasonable agreement. After optimal point prediction, minimum aflatoxin concentration below 0 µg/g was achieved at the predicted conditions (A = 30.42 ^{o}C, B = 10.58 days and C = 14.49%) except in AFG2 (3.33 µg/g). Aflatoxin production below the European Commission permissible limit in food (5 µg/kg) and feed (5-300 ppb) could be attained at actual conditions; A = 30 ^{o}C, B = 11 days, C = 15%.

Keywords: Aflatoxin, *Aspergillus parasiticus*, Wheat flour, Optimisation, RSM,

INHIBITION OF ASPERGILLUS AND PENICILLIUM FUNGI AND DEGRADATION OF AFLATOXINS BY OZONE TREATMENT DURING WHEAT AND MAIZE STORAGE

PETRENKOVA V.[1], TARAN G.[2], PUGACH S.[2], ZAMURIEV A. [2], OPALEV P.[2], YAROSHENKO M.[3].

[1]Plant Production Institute of National Academy of Agrarian Sciences of Ukraine, Kharkiv, Ukraine; [2]StreamOzone ltd., Kharkiv, Ukraine; [3]National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", National Academy of Agrarian Sciences of Ukraine, Kharkiv, Ukraine.

tarang@kipt.kharkov.ua

The problem of decreasing fungal contamination of a grain and consequentially decreasing the mycotoxins content in its volume during long-term storage is relevant worldwide. Therefore, the main objective of the experimental studies was to determine the degree of growth of micromycetes suppression with ozone at artificial contamination of wheat grains with Penicillium verrucosum and maize grains with Aspergillus flavus.

To solve this problem the corn grain artificially contaminated with Aspergillus flavus and the wheat grain infected with Penicillium verrucosum were treated for 120 hours once every 2 weeks in a 100-liter model silo. The ozone concentration in the mixture was 100 mg/m³. The measurements have shown that ozone inhibits fungal contamination and not only reduces its content in samples, but also significantly reduces the number of colonies per 1 g of grain (from 3 to 6 times in various samples of grain).

Monitoring the content of mycobiota at a long-term storage (for 6 months) of a batch of 1000 kg grain, some of the batch was additionally infected with Penicillium nordicum, was studied. The studies have shown that ozone inhibits fungal contamination during storage. The percentage of residual the dispute that survived of Aspergillus flavus averaged 25% (75% inhibited). For Penicillium nordicum - 40% (60% inhibited).

The effect of ozone on the Aflatoxins content in corn grain, additionally infected with a micromycetes Aspergillus flavus was studied. At a degree of contamination with a micromycetes more than 30*104 spores/g, the Aflatoxins content decreases by 2.5 times (by 60%). It's correlates with decreasing the content of fungal contamination in the studied samples. With low contamination of wheat and maize grains with micromycetes of Aspergillus flavus and Penicillium verrucosum (less than 104 spores/g), Aflatoxins (B1, B2, G1, G2) were not found (less than 0.002 mg/kg).

This research was carried out in frame of the MycoKey project funded by European Commission under Horizon 2020 research and innovation programme (GA # 678781).

THE TOXIGENIC ACTIVITY OF PHENOLIC COMPOUNDS IN TEA TREE, ROSEMARY, EUCALYPTUS AND LAVENDER ESSENTIAL OILS ON MYCOTOXINS PRODUCTION

PUVAČA N.[1], BURSIĆ V.[2], AVANTAGGIATO G.[3], VUKOVIĆ G.[4].
[1] *Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, Cvećarska 2, 21000 Novi Sad, Serbia;* [2]*Department for Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia;* [3]*Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Amendola, 122/O, 70126 Bari, Italy;* [4]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia.*
nikola.puvaca@fimek.edu.rs

Aim of this research was to determine phenolic compounds content of tea tree (*Melaleuca alternifolia* L.), rosemary (*Rosmarinus officinalis* L.), eucalyptus (*Eucalyptus oblique* L.), and lavender (*Lavandula angustifolia* L.) essential oils most used in food and feed industry, and their toxigenic activity on ochratoxin A production. Phenolic quantification was carried out by gas chromatography and gas chromatography-mass spectrometric analysis, while compound identifications were performed by comparison of their linear retention indices with standards and homemade library databases. The toxic activities of essential oils were evaluated by inhibiting *Aspergillus niger* production of ochratoxin A. The measurement of the toxin was performed by high performance liquid chromatography. In the presence of essential oils, the ochratoxin A production depended on the incubation temperature of 20 and 30°C. Our analyses have shown that the *M. alternifolia* essential oil is richest in terpinen-4-ol, *R. officinalis* and *E. oblique* essential oils in 1,8-cineole, and *L. angustifolia* essential oil in ethanol, 2-(2-ethoxyethoxy)-, also known as carbitol, respectively. It was also found that investigated essential oils could serve as the biocontrol inhibitors against contamination by ochratoxin A in the feed as a natural product.

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

to Index

NEW STRATEGY FOR THE INHIBITION OF MYCOTOXIGENIC FUNGI IN CEREALS DURING STORAGE

QUILES J.M.*, VILA P. V., LUZ C., CALPE J., MAÑES J., MECA G.
Laboratorio de toxicología, Departamento de Medicina Preventiva y Salud Pública, Ciencias de la Alimentación, Toxicología y Medicina Legal, Facultat de Farmàcia, Universitat de València. Av. Vicente Andrés, s/n, 46100 Burjassot, València (SPAIN).
Juan.quiles@uv.es

Isothiocyanates (ITCs) are antifungal substances characteristic of the plants of the Brassicaceae family. The aims of this study were to evaluate the antifungal properties of the bioactive compound allyl isothiocyanate (AITC) against *Aspergillus flavus* (8111 ISPA) AFs producer and *Penicillium verrucosum* (D-01847 VTT) OTA producer on corn, barley and wheat. The experiments were carried out initially in a simulated silo system for laboratory scale composed of glass jars (1L) containing 300 g of Barley and wheat (contaminated with *P. verrucosum*) and corn (contaminated with *A. flavus*). The cereals were treated with a disk of 12% hydroxyethylcellulose gel to which 500 uL of AITC was added and closed and incubated for 30 days at 21 °C. Next, simulated silos of 100 L capacity containing 70 Kg of cereals were used. Barley and wheat were contaminated under the same conditions as the previous trial. They were treated with a disc of 12% hydroxyethylcellulose gel to which 5 mL of AITC were added, they were closed and incubated for 90 days at 21 °C. The fungal growth of the inoculated fungi and the reduction in the formation of AFs and OTA were determined. The best results were obtained in the 1 L jars, where there was complete inhibition of fungal growth. In corn, the amount of AFB1 detected in the controls and the treated samples was 8.07 and 0.12 ppb, respectively. Likewise, in barley, the amount of OTA present in the controls and the treated samples was 0.28 and 0.09 ppb, respectively.

The research was supported by the European Project (H2020-Research and Innovation Action) MycoKey "Integrated and innovative key actions for mycotoxin management in the food and feed chain" GA 678781 and the Spanish Ministry of Science and Innovation (PID2019-108070RB-100).

EVALUATION OF TWO *TRICHODERMA* STRAINS AND THEIR CULTURE FILTRATE AGAINST MYCOTOXIGENIC FUNGI

STRACQUADANIO C. [1], MAGNANO DI SAN LIO G.[1], CACCIOLA S.O.[2], QUILES J.M[3]. G. MECA[3].

[1]*Department of Agricultural Science, Mediterranean University of Reggio Calabria, Localitá Feo di Vito, 89122-Reggio Calabria, Italy.* [2]*Department of Agriculture Food and Enviroment, University of Catania, V. S. Sofia 100, 95123-Catania, Italy.* [3] *Departemento de Medicina Preventiva, Facultat de Farmacia, Universitat de Valencia, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain.*

Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants, the antagonistic properties of which are based on the activation of multiple mechanisms. The aim of this study is to evaluate the ability of two *Trichoderma* strains (*T. asperellum* and *T. atroviride*) to inhibit the mycotoxigenic pathogens of the genus *Penicillium*, *Aspergillus* and *Fusarium*. The antagonistic ability was evaluated by dual culture assay. A plugs growing mycelium of pathogen and antagonist were placed, in opposite sides, in to a PDA plate and incubated at 25°C. The percentage inhibitory effect was assessed by comparing the growth rate of the pathogen with and without (control plates) *Trichoderma*. These strains of *Trichoderma* show good inhibition against all pathogens, obtaining values of 60-75%. Moreover, the inhibitory effect of the culture filtrated was investigated. *Trichoderma* was inoculated into sterilized PDB and incubated at 30°C for 30 days. The filtered culture was extracted with ethyl acetate (EtOAc) and it was tested against pathogens with disk diffusion test, when EtOAc extract was put in the surface of PDA plates with spores of pathogen and incubated at 25°C. After 48 h it was measured the inhibition zone. Also the Minimum Inhibitory Concentration and the Minimum Fungicidal Concentration (MIC & MFC) were determined. The extracts show MIC values between 0.09-0.78 and 0.39-3.13 mg/ml, and MFC values between 0.78-1.59 and 0.39-6.25 mg/ml for *T. asperellum* and *T. atroviride*, respectively.

ADVANCED GRAIN CLEANING SOLUTIONS FOR MYCOTOXIN REDUCTION

SLETTENGREN K.[1], PASCALE M.[2], MORANTES G.[1], REICHEL M.[3], KELF T.[1], LOGRIECO A.F. [2]

[1]Bühler AG, Uzwil, Switzerland; [2]Institute of Sciences of Food Production, National Research Council (ISPA-CNR), Bari, Italy; [3]Eurofins WEJ Contaminants, Hamburg, Germany.

katarina.slettengren@buhlergroup.com

In order to efficiently reduce the mycotoxin content in food and feed, a whole value chain approach is needed starting from Good Agricultural Practices to prevent contamination in the field, to control measures to prevent mycotoxin production during the storage period and throughout the processing line, until final consumption. Grain cleaning is the most effective post-harvest mitigation strategy to reduce high levels of mycotoxins due to the efficient removal of mold-infected grains and grain fractions with high mycotoxin content. Several studies have been performed during the last few years to investigate the reduction of various *Fusarium* toxins in maize, deoxynivalenol in wheat and barley, ergot in rye, and aflatoxins in peanuts and maize. Typical cleaning steps include (i) mechanical size separation and dust removal by aspiration, (ii) separation based on density differences, and finally (iii) optical sorting. Within grain milling, often a fourth cleaning step is included, (iv) "debranning", i.e. removal of the outer layers of the pericarp. These equipments can process up to 250 tons of grain per hour. Recently a completely new cleaning technology has been developed to reduce aflatoxins in grains based on the spectral properties of fluorescence. These well-proven and recent innovations for mycotoxin reduction will be further discussed in the presentation, taking a whole value chain approach.

This work was supported by the MYCOKEY project which has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 678781.

to Index

DETOXIFICATION OF FUMONISIN B1 BY *SPHINGOPYXIS* SP.FDS-1 AND ITS DEGRADATION MECHANISM

HU J.[1], LU H.[1], WANG G.[1] HOU M.[1], SHI J.[1,2], XU J.[1,2]*, KEY J.

[1]*Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base, Ministry of Science and Technology/Key Laboratory for Control Technology and Standard for Agro-product Safety and Quality, Ministry of Agriculture and Rural Affairs/Key Laboratory for Agro-product Safety Risk Evaluation (Nanjing), Ministry of Agriculture and Rural Affairs/Collaborative Innovation Center for Modern Grain Circulation and Safety/Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, P. R. China; [2]School of Food and Biological Engineering, Jiangsu Univeristy,Zhenjiang, Jiangsu, P. R. China*

xjh@jaas.ac.cn

Fumonisin B1 (FB1) is a water-soluble mycotoxin produced by Fusarium species, which is mainly found in maize products. It is highly toxic to livestock and is a potential carcinogen. In order to isolate FB1 degrading bacteria by using FB1 as the sole carbon source. First, we established a method for preparing large amount of toxins. We used a macroporous resin column combined with a high-speed countercurrent chromatography to separate FBs in a large quantity. The method yielded 1.55 g of FB1, 0.55 g of FB3 with purify of 96.8%, 95.6%, respectively from 1 kg rice culture, and the final yield of FBs in whole was 74.8%. Using FB1 as the sole carbon source, a bacterial strain FDS-1that can degrade FB1 was isolated from soil by enrichment culture procedures. FDS-1can degrade 50μg/mL FB1 in minimal medium within 4 days and the optimal temperature and pH for FB1 degradation are 25 °C, pH 9.0 respectively. Based on the sequence analysis of 16S rDNA combined with physiological and biochemical characteristics, strain FDS-1 was identified as *Sphingopyxis* sp. The results of FB1 degradation mechanism for *Sphingopyxis* sp.FDS-1 showed that the strain can transform FB1 to hydrolyzed FB1 and free tricarballylic acid by the intracellular esterase activity, and then hydrolyzed FB1 can be further transformed into a less polar compound lacking a fluorescamine-reactive amino group. The structure of this compound need to be isolated and identified.

This research was financially supported by the National Natural Science Foundation of China (31872914, U1604234), Jiangsu Agriculture Science and Technology Innovation Fund (CX(17)1003).

to Index

CRITICAL GOOD AGRICULTURAL PRACTICES REDUCE AFLATOXIN CONTAMINATION IN MAIZE IN HIGH RISK REGIONS

XU F.[1*], BAKER R.C.[1], WHITAKER T.[2], HARVEY J.[3],
ZHAO Y.[1], ZHANG G.[1], STEVENSON A.1
[1] Mars Global Food Safety Center, Beijing, China; [2] North Carolina State University, Raleigh, North Carolina, USA; [3] Kansas State University, Manhattan, Kansas, USA
Feng.Y.Xu@effem.com

Maize is consumed world-wide as a staple human food, livestock feed, and industrial raw material. However, it is susceptible to fungal attack and likely to be contaminated with aflatoxins under suitable conditions. Aflatoxin contamination of maize is a serious threat to human and animal health. Good Agricultural Practices (GAPs) form the basis of a "farm to fork approach" for prevention of aflatoxin contamination. We undertook a collaborative project between industry, governments, academia and NGOs to make GAPs more applicable and practicable. In this study we aimed to identify the critical GAPs that were most likely to reduce aflatoxin formation in maize grown in high risk regions, such as Africa, India, Thailand and other South-East Asia countries, with a specific focus on small-holder farmers. Five critical GAPs were identified and prioritized through consultation with 24 experts and a detailed literature review. These extended across the pre-harvest, harvest and post-harvest stages of maize cultivation, and were prioritized based on their likely impact on the level on the aflatoxin contamination. The five GAPs were: use of drought-tolerant varieties; early harvest before physiological maturity; sorting to remove damaged ears and those having poor husk covering; drying properly to 13-14% moisture content; storage in suitable conditions to keep the crop clean and with proper aeration. Further studies are required to validate these literature-based findings.

to Index

Session

11 November 2021

UNDISCLOSED, UNMET AND NEGLECTED CHALLENGES IN MYCOTOXIN EXPOSURE ASSESSMENT

FINK-GREMMLS J.
Utrecht University, Faculty of Veterinary Medicine, Yalelaan 104, 3584 CH Utrecht, The Netherlands.
J.Fink@uu.nl

Mycotoxin contamination of foods and feed and dietary exposure of animals to multiple mycotoxins has become a major concern for animal health and welfare. Any precise estimate of the rate of exposure is hampered by the broad variety of food and feed materials available on the global market and the increasing complexity of diets for individual animal species and age categories. Recent progress in analytical techniques allows the monitoring of feed materials for a broad spectrum of mycotoxins. The interpretation of such results, however, remains an unmet challenge, as predictive models for complex mixtures of mycotoxins as they occur in a mixed diet, are still not available. Moreover, the individual susceptibility of an animal or a herd depends on the overall health status, the co-prevalence of infectious diseases and the resilience of individual animals to multiple non-related external and internal stressors. Currently, one of the most prominent examples is the simultaneous exposure of animals (and humans) to mycotoxins and heat stress, associated with the global climate change. Heat stress in plants increase their susceptibility to fungal invasion and mycotoxin production, and heat stress in animals readily impairs the integrity of the intestinal barrier, which is also the target for many mycotoxins at those low levels, which are commonly found in controlled mixed feeds. In turn, the subsequent immunomodulation increases the risk for the spread of infectious diseases and comprises therefore an advanced persistent threat for animal welfare, health, and productivity.

THE INTESTINE A TARGET FOR MYCOTOXINS : WHAT CAN WE LEARNED FROM PIGS

OSWALD I.P.
Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France.
isabelle.oswald@inrae.fr

As the most exposed surface of the body, the intestinal mucosa faces significant chemical and biological challenges. The intestinal mucosa has three main physiological functions. It establishes a physical barrier between the internal environment and the luminal content. The intestinal mucosa is also responsible for the digestion of luminal nutrients and their subsequent absorption. The epithelium is at the interface of the immune system and luminal content, including food antigens and microbial products. This implies a regulation of local defense mechanisms which consists in integrating all the signals from the outside and inside world to preserve the equilibrium conditions of immune homeostasis. All these intestinal physiological functions can be targeted by food contaminants such as mycotoxins. Among the "regulated" mycotoxins, deoxynivalenol and fumonisins have been studied in particular for their toxicity on the intestine. They are not only locally toxic to this organ, but also disrupt many intestinal functions and modify the immune response. This results in systemic toxicity leading to numerous symptoms and an alteration of the zootechnical parameters. Contamination of food with mycotoxins also increases the translocation of bacteria in the intestine and therefore intestinal and systemic infections. A lot of these experiment have been performed in pigs. For aflatoxins, zearalenone and ochratoxins, less data are available on their intestinal toxicity. The increased performance of analytical methods reveals new toxins, especially emerging ones, as well as "masked" or "modified" forms; it remains to be determined the impact of these toxins on the intestine. Global surveys indicate that co-contamination occurs frequently, but the hi.ealth risk from exposure to a combination of mycotoxins is not fully understood.

COMPARATIVE TOXICOKINETICS OF MAJOR MYCOTOXINS IN FOOD-PRODUCING ANIMALS

CROUBELS S.*, ANTONISSEN G., DEVREESE M.
Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium,
siska.croubels@ugent.be

Several *Fusarium* mycotoxins are frequently detected food and feed contaminants on a worldwide basis. Some of these compounds are regulated in food and feed, others are considered more emerging mycotoxins. Besides mycotoxins as such, food and feed may be co-contaminated with modified forms thereof, such as *deoxynivalenol*-3-β-D-*glucoside* and zearalenone-14-glucoside.
To adequately assess internal exposure and *in vivo* toxicity, knowledge on the *in vivo* oral bioavailability, disposition and toxicokinetic properties in humans and animals is mandatory. Moreover, comparative insights in differences in absorption, distribution, metabolism and excretion (ADME) processes, might reveal species-specific differences in sensitivity towards the toxic effects. Hence, comprehensive toxicokinetic modeling, i.e. mathematical descriptions of all ADME processes, offers great tools in this research area.
The goal of this presentation is to provide an overview of major species-specific differences in oral bioavailability and toxicokinetic properties, with emphasis on *in vivo* biotransformation, between pigs and poultry. Selected examples of trichothecenes, zearalenone and enniatins will be given. Also specific differences in toxicokinetics between bird species such as broiler chickens and turkeys, will be presented. Since pigs are being promoted as potential superior animal model for humans, a brief comparison between toxicokinetic properties of some mycotoxins in pigs and humans will be presented as well.

Part of this research was financially supported by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey)

to Index

Mycotoxins in Animal Production 1

Human and Animal Health & Toxicology

THE EFFECTS OF MYCOTOXINS ON THE INTESTINAL MICROBIAL HOMEOSTASIS

ANTONISSEN G.[1,2], VAN IMMERSEEL F.[2], CROUBELS S.[1].

[1] *Department of Pharmacology, Toxicology and Biochemistry,* [2] *Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

gunther.antonissen@ugent.be

The dynamic crosstalk between intestinal epithelial cells, intestinal microbiota, local immune cells, and nutrition represents one of the major regulatory mechanisms to maintain intestinal homeostasis. Dysregulation of this homeostasis can result in increased inflammatory signaling, increased epithelial permeability, and dysbiosis of the microbiota, which are recognized to play a role in the pathophysiology of a variety of gastrointestinal disorders. Ingestion of low to moderate levels of mycotoxins can cause an array of metabolic, physiologic and immunologic disturbances, with the gastro-intestinal tract as one of the major target organs. Understanding these interactions of mycotoxins with the gastro-intestinal environment will be of major importance for future risk assessment and the organization of efficient mitigation strategies. Different studies have demonstrated an impact of mycotoxin exposure on the gut microbiota composition and diversity in humans and different animal species. It has been observed that the abundance of different bacterial families / genera such as *Lachnospiraceae*, *Ruminococcaceae*, *Lactospiraceae*, *Faecalibacterium*, *Clostridiaceae Enterobacteriaceae*, and *Roseburia* can be affected by mycotoxin exposure. Furthermore, the negative impact of mycotoxins on the GIT physiology and immune system might also enhance the animal susceptibility to the (opportunistic) pathogenic organisms present in the microbiota such as *E. coli*, *Salmonella*, and Clostridia. Finally, the intestinal microbiota also play a role in the metabolism of mycotoxins and their modified forms, and even the intestinal detoxification of mycotoxins.

GA is supported by a postdoctoral fellowship from the Research Foundation – Flanders (12V6418N). GA is appointed as chairholder of the Chair Poultry Health Science, jointly established by Ghent University, Vetworks and Poulpharm.

NATURAL ANTIOXIDANTS AS MITIGATORS OF AFB1 ADVERSE EFFECTS IN BOVINES : IN VITRO AND IN VIVO STUDIES

NEBBIA C.[1], GIROLAMI F.[1], GHADIRI S.[1], BADINO P.[1], DALL'ASTA C.[2], BARBAROSSA L.[3], MARTIGNANI E.[1], PAULETTO M.[4], DACASTO M.[4], ZAGHINI A.[3]

[1] Department of Veterinary Sciences, University of Turin, Largo Braccini 2, 10095 Gruglasco (Italy). [2] Department of Food and Drug, University of Parma, Via G.P. Usberti 27/A, 43124 Parma (Italy). [3]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Italy. [4] Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, I-35020 Legnaro (Padua), Italy;
carlo.nebbia@unito.it

The effects of different antioxidants (curcumin, curcuminoids, quercetin and resveratrol) were tested *in vitro* on bovine mammary gland (BME-UV) and fetal hepatocyte-derived (BFH12) cell lines, respectively.
Cell viability of AFB1-treated (96-750 nM) BME-UV cells was increased by antioxidants (5µM) in the order quercetin > resveratrol > curcuminoids, curcumin alone being poorly effective, while AFM1 synthesis (LC-MS/MS) was decreased to an extent matching their potency in contrasting the AFB1-dependent cytotoxicity.
BFH12 cells were exposed to 3.6 µM AFB1 and incubated with curcumin, curcuminoids or resveratrol (2.5–30 µM). Antioxidants caused a concentration-dependent increase in cell viability, along with a decrease in both lipid peroxidation (MDA) and AFM1 synthesis (LC-MS/MS). In addition, all antioxidants at their highest tested concentration, counteracted the AFB1-mediated increase in the activity of CYP3A, which is implicated in AFB1 bioactivation. Overall, curcumin and curcuminoids were efficacious at lower concentrations than resveratrol.
Moreover, the effect of turmeric powder (TP, 20g/head/day), a registered feed additive containing curcumin and curcuminoids, was examined in lactating Friesian dairy cows exposed for 10 days to dietary AFB1 concentrations at the maximum EU limits (5 µg kg/feed). AFB1-exposed cows showed i) AFM1 average milk levels slightly exceeding at some time-points the EU maximum tolerance levels (0.05 µg/L) and ii) a decrease in serum antioxidant capacity. No statistically significant differences occurred in TP-supplemented cows, despite a positive trend in the second part of the trial.
The low bioavailability of curcumin and curcuminoids from TP might explain the remarkable difference with the results from *in vitro* studies.

This research was financially supported by the Italian Ministry of University and Research - Research Project of National Interest (PRIN-2015NL8JWS)

MULTIPLE MYCOTOXIN EXPOSURE DURING PREGNANCY AND RISKS OF ADVERSE BIRTH OUTCOMES: A PROSPECTIVE COHORT STUDY IN RURAL ETHIOPIA

TESFAMARIAM K.[1,2,3], DE SAEGER S.[3], DE BOEVRE M.[3], LACHAT C.[1].

[1] *Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.* [2] *Department of Public Health, College of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia.* [3] *Center of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium. - MYTOX-SOUTH® coordination unit.*

KokebTesfamariamgent.be

Maternal mycotoxin exposure has been associated with adverse birth outcomes in low- and middle-income countries. The evidence, however, is inconsistent and mainly limited to the assessment of a single mycotoxin. We assessed biomarkers of exposure to multiple mycotoxins during pregnancy and the associations with adverse birth outcomes in rural Ethiopia. We analyzed data from 579 pregnant women before 24 weeks of gestation enrolled in a prospective cohort study. Mycotoxin exposures were determined using LC-MS/MS. Multivariable linear probability models were used to assess the associations between mycotoxin exposure and small for gestational age and preterm birth. We applied principal components analysis to reduce the dimensionality of biomarker data from several taxonomic mycotoxin groups. All pregnant women were co-exposed to at least five mycotoxins, and one pregnant woman was co-exposed to 27 mycotoxins. Fumonisins (FB), *i.e.*, FB2, FB3, FB1, and tenuazonic acid were the most frequently identified mycotoxins in 98.8%, 95.3%, 93.3%, and 81.4% of the samples, respectively. Deoxynivalenol was detected in 38.7%, nivalenol in 50.1%, ochratoxin α in 67.9%, and zearalenone in 50.9% of the serum samples. Although we did not find a statistical association between the mycotoxin biomarkers and adverse birth outcomes, the present findings indicate an extensive presence of multiple mycotoxins exposure among pregnant women in rural Ethiopia. Tentative findings regarding associations between chronic maternal aflatoxin exposure and poor fetal growth trajectories will be discussed in the presentation. Further research is needed to determine the thresholds for safe human mycotoxin exposure in relation to adverse pregnancy outcomes.

This research was financially supported by VLIR-UOS Network program, Ghent University (MYTOX-SOUTH® consortium), and NUFFIC (NICHE/ETH/179) Netherlands Initiative for Capacity Development in Higher Education.

THE EMERGING *FUSARIUM* MYCOTOXIN, 2-AMINO-14,16-DIMETHYLOCTADECAN-3-OL (AOD) A SPHINGANINE ANALOGUE, INDUCES MASSIVE CELLULAR VACUOLIZATION

SOLHAUG A.[1], TORGERSEN M.L.[2], HOLME J.A.[3], WIIK-NILSEN J.[1], THIEDE B.[4], ERIKSEN G.S.[1]

[1]Chemistry and Toxinology Research group, Norwegian Veterinary Institute, 0454 Oslo, Norway. [2]Department of Molecular Cell Biology, Institute for Cancer Research, the Norwegian Radium Hospital, Oslo University Hospital, 0379 Oslo, Norway. [3]Department of Environmental Health, Division of infection Control and Environment and Health, Norwegian Institute of Public Health, 0403 Oslo, Norway. [4]Department of Biosciences, University of Oslo, 0316 Oslo Norway

Anita.Solhaug@vetinst.no

The mycotoxin 2-Amino-14,16-dimethyloctadecan-3-ol (AOD), has been isolated from cultures of the fungus *Fusarium avenaceum*, one of the most prevalent *Fusarium* species. AOD is an analog of sphinganine and 1-deoxysphinganine, which are important intermediates in the *de novo* biosynthesis of cellular sphingolipids. AOD induced a transient accumulation of vacuoles in the human liver cell line HepG2. The effect was seen at non-cytotoxic concentrations and was not linked to cell death processes. Proteomic analyses indicated that protein degradation and/or vesicular transport may be a target for AOD. The AOD-induced vacuoles were lysosomal-associated membrane protein-1 (LAMP-1) positive, suggesting that they most likely originate from lysosomes, late endosomes or autolysosomes. Accordingly, both endosomal and autophagy protein degradation were inhibited. Further studies also revealed that the vacuolization is dependent of acidic lysosomes. Overall, the results strongly suggest that the increased vacuolization are due to an accumulation of AOD in lysosomes, late endosomes and/or autolysosomes thereby disturbing of the later stages of the endolysosomal process.

This research were funded by FUNtox, strategic initiative of the Norwegian Veterinary. Maria L Torgersen was funded by the Norwegian Research Council.

PROTECTIVE ROLE OF FERMENTED WHEY AND PUMPKIN EXTRACT AGAINST AFB1 AND OTA REVEALED BY OMICS

MANYES L.*, FRANGIAMONE M., CIMBALO A., ALONSO-GARRIDO M., FONT G.
Laboratory of Food Chemistry and Toxicology. Faculty of Pharmacy. Universitat de València. Carrer Vicent Andrés Estellés s/n. 46100 Bujassot. Spain.
lara.manyes@uv.es

Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are mycotoxins which widely contaminate cereals and cereal-based products. However, it has been demonstrated that the use of functional ingredients can reduce mycotoxins absorption and toxicity in both humans and animals. The aim of the study was to evaluate the effect of milk fermented whey (WF) and pumpkin rich in carotenoids against AFB1 and OTA cytotoxicity in lymphoblastomic and neuroblastomic human cell lines through an omic and biomolecular approach. For this purpose, cells were exposed to intestinal digests of bread contaminated with or without mycotoxins (low concentration AFB1 and OTA) and enriched with 20% of pumpkin and WF, during 7 days. A gel-free shotgun proteomic approach was employed to identify features across QTOF-LC/MS system in Jurkak T-cells. Bioinformatic analysis using DAVID platform showed the identification of proteins involved in several metabolic pathways, mainly in gluconeogenesis, antioxidant activity and nucleosome assembly. More specifically, histones' expression implicated in nucleosome assembly (H2A, H2B, H2C, H3 and H4) was increased when exposing cells to functional ingredients. Furthermore, repression of cyclin A2 associated to limiting the growth of carcinogenic cells, confirmed the preventive effect of these functional ingredients. Microscopy and flow cytometry were the techniques used with SH-S5YS cells evidencing changes in cell differentiation at dopamine and tubulin expression level.

This work was supported by the Spanish Ministry of Science and Innovation (PID2019-108070RB-I00-ALI) and (BES -2017-081328) and the Generalitat Valenciana (PROMETEO/2018/126).

VALIDATION OF BIOMARKER STANDARDS FOR MULTI-MYCOTOXIN EXPOSURE ASSESSMENT IN HUMAN POPULATIONS

MARK W. SUMARAH
London, ON, Canada. [3] *London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada.*
mark.sumarah@canada.ca

Exposure to the big five agricultural mycotoxins: aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol in food is a global concern. This is especially important in the developing world where populations can be exposed to multiple toxins simultaneously at orders of magnitude above tolerable limits. Mycotoxin exposure can be accessed indirectly by analyzing the mycotoxin concentration in the diet of individuals and extrapolating to predict human exposure. Toxins and their metabolic transformation products can also be measured directly in biological fluids such as urine or blood. In order to perform reliable multi-mycotoxin assessments in humans, validated biomarker of exposure are needed. Some of these analytical standards are commercially available while others require synthesis. We have refined and simplified the synthesis of unlabelled and isotopically labelled AFB1-lysine, AFG1 -lysine and DON-glucuronide and have made these standards available for research purposes. These standards were used to study aflatoxin exposure in Nigerian children with severe acute malnutrition (SAM) and we performed a multi-mycotoxin exposure assessment of Rwandan women of child bearing age. We are currently working with a number of groups to compare the available standards and methods for AFB1-lysine determination and on the production of a reference serum. Our goal is to improve data quality and reproducibility so that proper comparisons can be made between studies.

SENSITIVITY OF THE PORCINE HEPATIC CELL MEMBRANE TO FUMONISIN B1 DURING SHORT EXPOSURE PERIOD

ALI O.[1], SZABÓ-FODOR J.[2], FÉBEL H.[3], MÉZES M.[4], BALOGH K.[4], GLÁVITS R.[5], MKOVÁCS M. [1,2], ZANTOMASI A.[6] AND SZABÓ A[1,2].

[1]Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Physiology and Animal Health, Kaposvár Campus, 7400 Kaposvár, Hungary. [2]Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Physiology and Animal Health, MTA-KE-SZIE Mycotoxins in the Food Chain Research Group, Kaposvár Campus, 7400 Kaposvár, Hungary. [3]Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Nutrition and Nutrition Physiology, 2053, Herceghalom, Hungary. [4]Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Feed Safety, Gödöllő Campus, 2103 Gödöllő, Hungary. [5]Autopsy Ltd., Telepes u. 42, 1147 Budapest, Hungary. [6]Department of Animal Science, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy.

omeralfaroug.ali@gmail.com

The hepatic membrane fatty acid profile and oxidative capacity to fumonisin B1 (FB_1) was examined *in vivo* in piglets (n=7 piglets/group fed an artificially contaminated diet (20 mg FB_1/kg) for 10 days. The FB_1-induced-hepatotoxicity was combined with more significant alterations in the total phospholipid (TPL) FA profile than the triglyceride (TG) FA profile. In the TPL FA profile, pronounced alterations were in the polyunsaturated FAs (PUFAs); decreased omega-3 FAs (docosapentaenoic and docosahexaenoic acids), and increased arachidonic acid. These alterations led to a higher omega-6:omega-3 ratio; a similar finding was noted in TGs. There was no alteration in total saturation or total monounsaturation. Malondialdehyde and antioxidant enzymes (reduced glutathione and glutathione peroxidase) were higher in the FB1-group livers. When the plasma enzymes were examined, only the alkaline phosphate level increased in response to FB_1-exposure. Thus, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (GGT) remained unaltered. Alterations in the FA, oxidation level and plasma enzymes were related to vacuolar degeneration of the hepatocytes' cytoplasm, but it was not severe. Additionally, this study revealed that administering the FB_1 dose for 10 days had no adverse effect on piglet growth performance or feed efficiency. In conclusion, our study illustrates the sub-acute adverse effects of FB_1 on the liver over a shorter period. Furthermore, our findings indirectly imply that FA desaturase enzymes and ceramide synthases have been disrupted.

This research was financially supported the GINOP-2.2.1.-15-2016-00046 and the EFOP-3.6.3.-Vekop-16-2017-00005 projects, Hungary.

COMPREHENSIVE TOXICOKINETIC ANALYSIS REVEALS AGE-RELATED DIFFERENCES IN SYSTEMIC EXPOSURE TO DEOXYNIVALENOL AND ZEARALENONE MODIFIED FORMS IN THE JUVENILE PIG AS A HUMAN PAEDIATRIC SURROGATE MODEL

A. CATTEUW[1], S. DE BAERE[1], G. ANTONISSEN[1], L. IVANOVA[2], S. DE SAEGER[3], M. DE BOEVRE[3], M. DEVREESE[1], S. CROUBELS[1].

Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. [2]Chemistry Section, Norwegian Veterinary Institute, Ullevålsveien 68, 0454 Oslo, Norway. [3]Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium.
Amelie.Catteuw@UGent.be

The EFSA CONTAM Panel recommends to perform toxicokinetic studies in order to reduce the uncertainties associated with the current risk assessment concerning modified deoxynivalenol (DON) and zearalenone (ZEN) in humans and animals [1,2]. One of those major uncertainties is the possible higher systemic exposure of juveniles, one of the most vulnerable population groups in both humans and animals concerning xenobiotics. Especially during weaning the intestinal barrier and immune system are compromised. Consequently, the goal of this study was to unravel age-related differences in toxicokinetic processes of DON-3-glucoside (DON3G), ZEN-14-glucoside (ZEN14G) and ZEN-14-sulfate (ZEN14S) in the weaned piglet (4-weeks-old) as a human paediatric surrogate model. Two studies were each conducted on eight healthy piglets. Single equimolar boluses of DON3G (55.7 µg/kg BW) and DON (36 µg/kg BW) or ZEN (331 µg/kg BW), ZEN14G (500 µg/kg BW) and ZEN14S (415 µg/kg BW) were administered by intravenous and oral administration, following a double two-way cross-over design. Blood and urine were sampled at different time point post-administration and plasma/urine concentrations of DON, DON3G, ZEN, ZEN14G, ZEN14S and their phase I and II metabolites were quantified using validated LC-MS/MS and LC-HRMS methods. Data were processed using tailor-made compartmental models. Results were statistically compared to results from toxicokinetic studies in older pigs (8-11-weeks-old)[3,4]. Results revealed significant age-related differences in toxicokinetic processes and subsequent higher systemic exposure to DON and ZEN of weaned compared to older pigs, particularly reflected by the significantly higher absorbed fraction of DON3G (83% vs 16%) and ZEN14G (94% vs 61%).

This work was supported by Horizon 2020 (H2020-MYCOKEY-GA 678781).

(1) EFSA (2017). *EFSA J.* 15(9), 4718. (2) EFSA (2017). *EFSA J.*15(7), 4851. (3) Broekaert *et al.* (2017). *Arch. Toxicol*. 91 (2), 699–712. (4) Catteuw *et al.* (2019). J. Agric. Food Chem. 67 (12), 3448-3458.

EFFECTS OF DIETARY CHRONIC EXPOSURE TO AFLATOXINS ON PRODUCTION, REPRODUCTION AND EMBRYONIC DEVELOPMENT OF LAMBARI FISH (ASTYANAX ALTIPARANAE).

M.P. CHAGURI[1], S.H.S. GODOY[1], R.M. BERTOLINI[2], N.R.A. ROCHA[1], G.E. ROTTINGHAUS[3], G.S. YASUI[4], R.L.M. SOUSA[1], A.M. FERNANDES[1].

[1]Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte, 225, CEP 13635-900, Pirassununga, Brasil. [2]Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista, R. Prof. Dr. Antônio Celso Wagner Zanin, 250 - Distrito de Rubião Junior, CEP 18618-689, Botucatu, Brazil. [3]Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, 901 E. Campus Loop, 65211, Columbia, United States of America. [4]Centro Nacional de Pesquisa e Conservação de Peixes Continentais – ICMBio-CEPTA, Rodovia SP-201, Km 7,5, CEP 13.630-970, Pirassununga, Brasil.

mpchaguri@hotmail.com

The aim of this research was to evaluate productive and reproductive parameters in lambari fish (*Astyanax altiparanae*) long term exposed to aflatoxins in the diet. Lambari fish were fed diets witho, 10, 20 and 50 µg aflatoxins/kg for 120 days and fertilization procedures were performed by the semi-natural reproduction process. Parameters evaluated included feed intake, final weight, weight gain, mortality, gonadosomatic index (GSI), semen quality, oocyte volume, fertilization rate and embryonic development of F1 generation. Regarding productive parameters, fish fed 50 µg/kg presented the worst indexes. Reproductive parameters showed that aflatoxins influenced the gonadosomatic index (GSI) of females, with lower values in females consuming contaminated feed, while males did not present differences. It was not observed variation in semen quality among treatments. However, females exposed to aflatoxins had lower oocyte volume and fertilization rate. Females fed 50 µg/kg had the worst reproductive rates. Lambari fish exposed to aflatoxins showed alterations in the embryonic development of F1 generation. A higher number of unfertilized eggs (UFE) was observed in treatments exposed to aflatoxins, and fish fed 50 µg/kg presented the highest percentage of UFE (47.3%). Treatment 10 µg/kg presented the largest number of abnormal larvae when compared to the other treatments. The results showed that the aflatoxins limits required by regulatory agencies in feed are not enough for avoiding productive and reproductive losses in lambari fish. Therefore, monitoring and efforts to reduce aflatoxin exposure should be considered to avoid productive and reproductive concerns in lambari fish.

This research was financially supported by the Project 2017/15110-7 (Fundação de Amparo à Pesquisa do Estado de SãoPaulo, Brasil).

DYNAMIC EVOLUTION OF BACTERIAL, YEAST AND FUNGAL COMMUNITIES DURING ENSILING OF ALFALFA SILAGE AND AFTER EXPOSURE TO AIR.

A. GALLO[1], F. GHILARDELLI[1], M. CAMARDO LEGGIERI[2], S. FODIL[2], P. BATTILANI[2]

[1]Department of Animal Science, Food and Nutrition (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, ITALY. [2]Department of Sustainable Crop Production (DIPROVES), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, ITALY.

The aim was to evaluate dynamic evolution of bacterial, yeast, fungal communities and mycotoxin production during ensiling of alfalfa silage and after silage air exposure. A fermentation-kinetic study was performed on alfalfa, harvested at 3/10 bloom, mowed, wilted, windrowed and ensiled on June 8, 2018. Chopped silages were placed into 20 L plastic jars at a density of 113±2.6 kg DM m^3. Mini-silos were opened at different ensiling times and samples were analyzed for fermentation parameters and microbiological populations (colony forming units/g) after 0 (before ensiling), 1, 2, 3, 7 and 14 days of ensiling. Mycotoxins and fungal occurrence (cfu/g), with focus on *Fusarium*, *Penicillium*, *Aspergillus* spp. were quantified after 0, 2, 7 and 14 days of ensiling. On the alfalfa mini-silos opened after 14 days, an aerobic stability test was carried out and aliquots of air exposed silage were collected for bacterial and fungal community characterization or mycotoxin contamination in specific days. During ensiling, both homofermenter LAB and heterofermentative bacteria appeared dominant whereas molds, yeasts, *Enterobacteriaceae* were inhibited. No *Acetobacter* spp. was counted. *Aspergillus*, *Fusarium* spp. and other fungi increased after 2 days of ensiling and then decreased. On the contrary, *Penicillium* spp. remained stable during ensiling phases. Among mycotoxins, DON increased up to about 562 µg/kg DM with ensiling, whereas the other *Fusarium* produced mycotoxins remained constants. When exposed to air, molds, yeasts, Enterobacteriaceae, *Acetobacter*, *Fusarium* and *Penicillum* spp. grown with different dynamic behaviors and mychopenolic acid concentration increased up to 2423 µg/kg DM, whereas *Aspergillus* spp. decreased.

THE USE OF BLOOD BASED BIOMARKERS IN THE EVALUATION OF MYCOTOXIN CONTAMINATED PIGS

KOPPENOL A.[1], GIACOMINI L.[2] AND INGBERMAN M.[3].
[1]Impextraco NV, Wiekevorstsesteenweg 38, 2220 Heist-op-den-Berg, Belgie. [2]Instituto de Solucoes Analiticas Microbiologicas e Technologicas Ltda. (SAMITEC) Rod. RSC 287, nr 2800, Santa Maria, Brazil. [3]Imunova Analises Biologicas Ltda, Rua Imaculada Conceicae, 1430 Technoparque da PUC, Curitiba, Brazil.
astrid@impextraco.be

In total 36 pigs were housed during 42 days and assigned to 1 out of the 3 treatments; (1) control diet, (2) contaminated diet with 50 ppm fumonisins, or (3) contaminated diet supplemented with a mycotoxin eliminator at 0.5% in a randomized design 3T*6R*2. Zootechnical parameters were determined weekly. Blood was sampled from 8 animals per treatment for blood biochemistry and flow cytometry. Contamination levels of fumonisins in the final diet were confirmed by HPLC analysis and both contaminated groups reached the objective of 50 ppm FB1+FB2. Pigs body weight (p=0.0019) and feed intake (p=0.0078) were significantly lower for the contaminated groups compared to the control group, resulting in significantly higher FCR (p=0.0080). The relative weight of the lungs was significant higher for the contaminated pigs compared with control pigs (p=0.0007). The total amount of circulating leucocytes (p=0.1355) and monocytes (p=0.0282) were reduced in the contaminated pigs, compared to the control pigs. Circulating T-lymphocytes were increased in contaminated pigs compared to controls (p=0.3458). An increase was found for total protein (p=0.1045), AST (p=0.0001) and GGT (p=0.0003) levels in the serum. The SA/SO ratio, known as the best biomarker for fumonisins, also significantly increased in the contaminated animals compared to the control pigs (p<0.0001). Animals supplemented with the mycotoxin eliminator showed intermediate results for all measured parameters, indicating not only the efficacy of Elitox® in eliminating the harmful effect of fumonisins on pig performance and health, but also the use of blood based biomarkers to evaluate the efficacy of mycotoxin eliminating feed additives.

PHOTOCATALYTIC INACTIVATION MECHANISM OF THE HYPERTOXIC SITE (C8=C9) IN AflATOXIN B1.

JIN MAO[1], QI ZHANG[1], ZHAOWEI ZHANG[1],
XIAOQIAN TANG[1] AND PEIWU LI[1]
[1]*National Reference Laboratory for Agricultural Testing P.R.China, Key Laboratory of Detection for Mycotoxins (Ministry of Agriculture), Laboratory of Quality & Safety Risk Assessment for Oilseed Products (Ministry of Agriculture),Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.*
maojin106@whu.edu.cn

Aflatoxin B1 (AFB1) is regarded as a main pollutant with high toxicity, carcinogenicity and teratogenicity in feed, and the double bond (C8=C9) of its terminal furan ring is the key hypertoxic site. Although semiconductor photocatalysis has been proposed to be a potential way of reducing or inactivating the toxicity of AFB1, the reaction mechanism of reactive oxygen species with the hypertoxic site has not been defined so far. Therefore, we designed a kind of all-solid-state Z-schematic composite by depositing CdS on the surface of WO_3 and uncovered the photocatalytic inactivation mechanism of the hypertoxic site in AFB1. On the bases of high resolution mass spectrum, radical trapping test and ^{18}O isotope-labeling studies, it can be concluded that the preferentially inactivating the C8=C9 site by the addition reaction of hydroxyl radical is the main pathway for the detoxification of aflatoxin B1. Furthermore, density functional theory (DFT) calculations were applied to reveal the reaction mechanism and verify that the hydroxyl radicals were most likely to react with the C9 site, and then form AFB1-9-hydroxy. These findings provide in-depth insights into the inactivation mechanism of hypertoxic site in AFB1, and the design of efficient photocatalysts for alleviating the risk of toxic pollutants.

This research was financially supported by the Natural Science Foundation of China (31871900, 31401601), National Key Project for Agro-product Quality & Safety Risk Assessment, PRC (GJFP2018001), International Science & Technology Cooperation Program of China (2016YFE0112900).

TOWARDS RISK ASSESSMENT FOR CITRININ: COMBINING OCCURRENCE, TOXICOKINETIC AND TOXICITY DATA TO ESTIMATE THE RISKS FOR HUMAN AND ANIMAL HEALTH.

MEERPOEL C. [1,2], VIDAL A.[1], HUYBRECHTS B.[3],TANGNI E.K.[3], CROUBELS S. [2], DEVREESE M.[2] AND DE SAEGER S.[1]

[1]Ghent University, Faculty of Pharmaceutical Sciences, Department of Bioanalysis, Centre of Excellence in Mycotoxicology and Public Health, Ottergemsesteenweg 460, 9000 Ghent, Belgium. [2]Ghent University, Faculty of Veterinary Medicine, Department of Pharmacology, Toxicology and Biochemistry, Salisburylaan 133, 9820 Merelbeke, Belgium. [3]SCIENSANO, Leuvensesteenweg 17, 3080 Tervuren, Belgium
Celine.Meerpoel@UGent.be

According to the European Food Safety Agency (EFSA), the risk assessment for citrinin (CIT) is incomplete, due to a lack of occurrence and toxicity data. Therefore, more data on occurrence, toxicokinetic characteristics and toxicity were gathered. First, CIT occurrence was investigated after analysis of feed (n = 90) and multiple foodstuffs (n = 427). In feed, CIT was detected in 50% of the analysed samples, in concentrations ranging from below the limit of quantification (LOQ) to 3.9 µg/kg. Regarding food, CIT was present in 42% of the samples in concentrations between <LOQ and 1,787 µg/kg. Cereals, herbs, meat and vegetarian foods were the most important group for CIT contamination. Then, toxicokinetic studies were performed and a porcine toxicokinetic model was built and extrapolated to humans. Furthermore, toxicokinetic parameters were estimated in broiler chickens, to investigate interspecies differences. Oral bioavailability for CIT was complete for broilers (113 – 131 %), while ranging from 37 to 44 % in pigs. CIT was more rapidly absorbed in pigs (T_{max} = 0.92 h) compared to broiler chickens (T_{max} = 7.33 h), but the elimination of CIT was slower in pigs ($T_{1/2el}$ = 26.81 h) compared to chickens ($T_{1/2el}$ = 1.97 h), due to the striking difference in clearance (Cl_{iv} = 9.87 mL/h/kg for pigs versus Cl_{iv} = 863.09 mL/h/kg for broilers). These data indicate major interspecies differences, also implying variances in risks of CIT for different species. Taking into account these data, a risk assessment was performed for both humans and animals.

This research was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 16/6308 (CITRIRISK)

DO WE UNDERESTIMATE THE POTENTIAL RISK OF FUSARIUM-DERIVED MYCOTOXINS

B. NOVAK[1], V. RAINER[1], I. TASCHL[2], G. SCHATZMAYR[1], E. MAYER[1]
[1]*BIOMIN Research Center, Technopark 1, 3430 Tulln an der Donau, Austria,* [2]*BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria.*
E-mail: barbara.novak@biomin.net

Fusarium fungi produce well-studied mycotoxins, such as deoxynivalenol (DON), zearalenone (ZEN) and fumonisins, whose maximum concentration is regulated in food and feed in European countries. Due to climatic changes and modern analytical systems, scientific community and regulatory authorities are becoming more aware about an increase of other uncommon *Fusarium*-derived compounds, so-called emerging mycotoxins. Currently, those are neither regulated nor closer described, and hence, might pose a risk for human and animal health.
In 2014, BIOMIN started their own survey to assess the mycotoxin contamination level of feed samples all over the world. Thereby, it has been seen that the prevalence of those emerging mycotoxins was rather underestimated compared to mycotoxins, known to be of agricultural relevance, such as DON or ZEN. Enniatin B and B1 as well as aurofusarin and culmorin occur in more than 79% of 1,141 pig feed samples, whereas DON and ZEN were only found in 77% and 73% of all samples, respectively. Even the median concentrations were partly higher than the ones of DON and ZEN. Therefore, more than 25 different fungal metabolites were investigated for their effect on an intestinal porcine cell line (IPEC-J2). Detrimental *in vitro* effects on cell viability and/or gut barrier integrity were determined for the cyclic depsipeptides enniatins (A, A1, B and B1), beauvericin, aurofusarin, apicidin and several other *Fusarium* derived mycotoxins. Since one sample is mostly co-contaminated with more than 10 different fungal metabolites, the establishment of appropriate methods in the living organism is required to investigate possible synergistic effects.

This research received funding from the Austrian Research Promotion Agency (Österreichische Forschungsförderungsgesellschaft FFG (grant numbers 853863 and 859603), as well as EFREtop (grant number 864743).

CAN GHRELIN INJECTIONS COUNTERACT THE DON-INDUCED REDUCED FEED INTAKE IN PIGLETS

SCHAUERHUBER C.[1], WENDNER D.[1], EMSENHUBER C.[1], GRUBER C.[2], WEIN S.[1], SCHATZMAYR G.[1] AND MAYER E[1].
[1]*BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria.* [2]*BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria*
cornelia.schauerhuber@dsm.com

Deoxynivalenol (DON) occurrence in feed is an emerging challenge for piglets, as this mycotoxin is known to reduce feed intake. The role of plasma ghrelin levels to counteract this negative effect of DON was assessed in a piglet feeding trial. Within our four experimental groups with six animals each, two groups received either DON contaminated feed (5 mg/kg) (group 1 and 2) or control feed (group 3 and 4), with either saline (1, 3) or ghrelin intravenous injection (2, 4). After an adaptation phase of 13 days, the animals were individually held and received their group specific feed for the experimental phase (five days). Ghrelin or saline injection (twice daily) and blood sampling (each prior to injection, variably scheduled post injection) were done via implanted central venous catheters. Following injection, feed was provided and feed intake was measured after 120 min. Saline injected groups showed plasma ghrelin concentrations below the LOD. Highest ghrelin concentrations were detected 15 min after injection, followed by a time-dependent decrease. The mean weight gain and the total feed intake were significantly lower in the DON-fed animals (1, 2) compared to the animals fed control diet + ghrelin (4). Ghrelin injections had numerically positive effects on weight and feed intake in both diets. Concluding, this trial showed that increased plasma ghrelin levels tend to counteract the DON-induced reduced feed intake. Its underlying mode of action could therefore be connected to the ghrelin pathway.

IN VIVO EVIDENCES ON THE PRESENCE OF MODIFIED AFLATOXIN IN ALMONDS INOCULATED WITH *ASPERGILLUS FLAVUS* IN A PIGLET ANIMAL MODEL.

M. SOLFRIZZO[1] L. GAMBACORTA[1], G. PERRONE[1], E. FICHANT[2], D. LACROIX[2], S. CROUBELS[3]

[1]*Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Italy*
[2]*Laboratoire d'Hormonologie, C.E.R. Groupe-Département Santé, Marloie, Belgium*
[3]*Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*
e-mail: michele.solfrizzo@ispa.cnr.it

We previously demonstrated that almond syrup preparation process produced an important increase of aflatoxins as compared to initial aflatoxin levels. It was supposed that water infusion of almonds activates almond enzymes that release free aflatoxins from modified (hidden or masked) aflatoxins. In this study groups of weaned piglets received single doses of AFB_1 standard as compared to groups of piglets that received fungal almond culture containing the same dose of free AFB_1 plus modified AFB_1. Urinary aflatoxin M_1 concentrations were measured and used as quantitative biomarker of AFB_1 intake. This analysis serves to check the possible *in vivo* conversion of modified AFB_1 into free AFB_1 in the gastrointestinal tract of piglets. A two-way cross-over toxicokinetic study was conducted with 8 healthy conventional male piglets. Inoculated almonds containing 90.5 ± 5.1 µg/g free AFB_1 and 9.1 ± 0.5 µg/g free AFB_2 plus modified forms, were slurried and given by gavage to piglets that received 1290 µg AFB_1 and 90 µg AFB_2 each. Each control piglet received a slurry of blank almond spiked with 1290 µg AFB_1. Urine was collected until 24 h post administration and volumes were carefully recorded for mass balance calculation. Urine samples were digested with β-glucuronidase, diluted with water and directly analyzed for AFM_1 by HPLC-FLD. The mean amount of AFM_1 excreted in 24 h by each piglet administered with 1290 µg AFB_1 standard was 34.02 µg, whereas the mean amount of AFM_1 excreted by each piglet administered with inoculated almonds was 42.55 µg (+25%). These results suggest that *A. flavus* growth on almonds can lead to produce free and modified AFB_1 (hidden or masked) and that piglets are able to release free AFB_1 from modified AFB_1 which is demonstrated by the increase of urinary AFM_1 as compared to piglets given only AFB_1 standard.

This work has been supported by the MYCOKEY project "Integrated and innovative key actions for mycotoxin management in the food and feed chain" (H2020 - Grant Agreement No 678781).

MYCOTOXIN EXPOSURE ASSESSMENT OF RWANDAN WOMEN OF CHILDBEARING AGE

COLLINS SL [1,2], WALSH J.P.[3,4], RENAUD J.B.[3], MCMILLAN A.M.[1,2], MILLER J.D.[5], REID G.[1,2] AND SUMARAH M.W [3,4].

[1] Centre for Human Microbiome and Probiotics, Lawson Health Research Institute, London, ON, Canada. [2] Department of Microbiology and Immunology and Surgery, University of Western Ontario, London, ON, Canada. [3] London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada. [4] Department of Chemistry, University of Western Ontario, London, ON, Canada. [5] Department of Chemistry, Carleton University, Ottawa, ON,

mark.sumarah@canada.ca

Mycotoxin contamination of crops in developing countries has a major impact on human health. Using a high-resolution LC-MS/MS approach with stable isotope dilution, we examined the impact of exposure on the health of Rwandan women of childbearing age. Serum and urine were analyzed from a cohort of 139 Rwandan women for the presence of aflatoxin, fumonisin, OTA, zearalenone and DON. AFB1-lysine was detected in 85% of serum samples at a mean concentration of 2.2 ± 1.8 pg/mg albumin. AFM1, AFB1, and AFG1 were detected in 47%, 8% and 26% of urine samples at concentrations of 98 ± 145, 9.1 ± 6, and 38 ± 80 pg/mg creatinine, respectively. FB1, FB2 and OTA were also detected in the urine of 30%, 15% and 71% of individuals in this population, at mean concentrations of 9.0 ± 18, 52 ± 40 and 24 ± 31 pg/mg creatinine, respectively. DON exposure was detected in 81% with 15-DON-GlcA the most frequently detected (55%), at a mean concentration of 21.7 ± 48.7 ng/mg creatinine. ZEA exposure was detected in 80% with α-ZOL the most frequently detected ZEA marker (61%), at a mean concentration of 8.9 ± 13.1 ng/mg creatinine. In this study, pregnant women had consistently greater concentrations of AFB1-lysine across each trimester compared to their non-pregnant counterparts. Given the exposures to multiple mycotoxins by this population, including to toxins above the PMTDI, improvements to food processing and storage are needed for this high-risk population.

HUMAN MYCOTOXINS INTERVENTION TRIAL: A STANDARDIZED PROTOCOL.

L. VISINTIN[1], M. DEBOEVRE[1], AND S. DESAEGRE[1].
[1]*Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, B-9000 Ghent, Belgium.*
lia.visintin@ugent.be

Mycotoxin exposure is known to contribute to adverse human health outcomes, however, data regarding human exposure remain lacking. Understanding the health consequences in humans requires the capability of conducting accurate exposure assessments of mycotoxins at individual level. The physiological compartments involved in mycotoxins transformation, absorption, and excretion (ADME-principle) are the kidneys, liver, and gastrointestinal tract. The full mycobolome, identified in urine, blood, and feces, forms the basis for a toxicokinetic model built by applying state-of-the-art modeling schemes. The standardized study protocol will be applied in the ERC project HuMyco providing a holistic procedure to unravel IARC group III-classified mycotoxin biomarkers of exposure through a human intervention trial. The protocol aims to identify new biomarkers of exposure, determine the ADME-toxicokinetic properties of the mycotoxins considered, and build a toxicokinetic profile-based model. The clinical trial will emphasize implementing non- and minimally-invasive sampling strategies. The main aspects of ethical considerations, informed consent, recruitment of the volunteers, and study settings of the intervention trial will be explained with a focus on the sample collection. Information about sample preparation, analysis, and data elaboration will be provided as well. The combination of results, involving the different matrices, improves the model's predictivity and highlights the superiority of multi-matrix approaches in biomarker detection and pathway elucidation. The harmonization of the protocol improves the reliability, repeatability and reproducibility of the experimental results. The obtained data offers public health and decisional authorities the opportunity to define specific legislation based on the risk for the population.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 946192, HUMYCO).

to Index

Session S5

Functional Genomics of Toxigenic Fungi

15 YEARS OF FUSARIUM GENOMICS: 2006-2021

WAALWIJK C.
Wageningen, The Netherlands
cees.waalwijk@wur.nl ; c.waalwijk@upcmail.nl

When the *Neurospora* genome sequence was presented at the XXIst Fungal Genetics Conference in Asilomar in 2001, excitement fled through the fungal community and the room was too small for the audience. If this would only be possible for agronomically important fungi like Fusarium. In a community approach this was initiated in 2004 and finally resulted in a 45-coauthored publication in Science in 2007: the *Fusarium graminearum* genome contains four chromosomes with a link between localized polymorphism and pathogen specialization (Cuomo, 2007). This opened up the avenue towards novel gene discovery and alternative splicing, while concerted expression profiles revealed/confirmed biosynthetic gene clusters (BGCs).
Subsequent genome sequences from other fusaria initiated comparative analyses within and between species of the genus. (Loss) of synteny between genomes revealed a large plasticity of Fusarium genomes including compartmentalization between slow and fast evolving genomic segments, a.k.a. two-speed genomes. These fast evolving (non-conserved) regions with generally low expression levels (Zhao, 2014) can be considered the genetic playground of the fungus allowing for elevated mutagenesis providing the flexibility to adapt to a (changing) environment and/or different host.
In the last 1½ decade, many of the underlying mechanisms for these phenomena have been uncovered, including horizontal gene transfer, sexual recombination, repeat induced point mutations (RIP), conserved expression of all genes involved in a particular biosynthetic pathway through "master"-regulators.
The comparative genomics approach has been a powerful tool to address many of the questions. However, a pangenomic strategy seems to be the way forward to identify core, accessory and unique gene sets among hundreds of isolates. Pantools will also be helpful in coping with the enormous amount of genome data that are emerging and in exchange within the Fusarium community.
Several of these findings with emphasis on *Fusarium* and (diversification and regulation of expression of) its mycotoxins will be discussed.

THE BZIP-TYPE TRANSCRIPTION FACTOR FVATFA REGULATES FUMONISIN, BIKAVERIN AND CAROTENOID PRODUCTIONS IN THE MAIZE PATHOGEN *FUSARIUM VERTICILLIOIDES*

SZABÓ ZS.[1], PÁKOZDI K.[1], EMRI T.[1], LEITER É.[1], LOGRIECO A.F.[2], HORNOK L.[3], PÓCSI I.[1*]

[1]*University of Debrecen, Department of Microbial Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, H-4032 Debrecen, Egyetem tér 1., Hungary;*
[2]*Institute of Sciences of Food Production (ISPA-CNR), 70126 Bari, Via Amendola 122/O, Italy;*
[3]*Szent István University, Faculty of Agricultural and Environmental Sciences, H-2103 Gödöllő, Páter Károly utca 1., Hungary.*
pocsi.istvan@science.unideb.hu

The deletion of the *Schizosaccharomyces pombe atf1* and *Aspergillus nidulans atfA* orthologue *FvatfA* gene in *F. verticillioides* resulted in various phenotypes. The *ΔFvatfA* strain showed (i) slower growth on Czapek-Dox Agar and Potato Dextrose Agar media, (ii) reduced invasive growth on unwounded tomato fruits, (iii) female sterility, (iv) conidia with shorter arc lengths (v) increased sensitivity to oxidative (H_2O_2, *tert*-butyl hydroperoxide, menadione sodium bisulphite) and cell wall integrity (Congo Red) stresses, (vi) defected fumonisin production, (vii) increased bikaverin yields and (viii) considerably decreased light-induced carotenoid production in comparison with both the wild-type and genetically complemented strains. The inability of the fungus to produce fumonisins was explained with the down-regulation of the *fum1* and *fum8* genes encoding the fumonisin polyketide synthase and α-oxoamine synthase in the fumonisin biosynthetic pathway, respectively. Similarly, the decreased carotenoid production was coupled to the down-regulation of two important genes, *carRA* (bifunctional gene encoding phytoene synthase and carotene cyclase) and *carB* (coding for carotene desaturase) in carotene biosynthesis. Interestingly, the increased bikaverin yields were not accompanied by the up-regulation of the bikaverin polyketide synthase gene *bik1* reflecting an indirect regulatory function of FvAtfA in this case, *e.g. via* modulating primary metabolic pathways of the fungus.

The research was financed by the European Union and the European Social Fund through the project EFOP-3.6.1–16–2016-00022, by the National Research, Development and Innovation Office (Hungary) K112181 and K119494 and by the Higher Education Institutional Excellence Program (NKFIH-1150-6/2019) of the Ministry of Innovation and Technology in Hungary, within the framework of the Biotechnology thematic program of the University of Debrecen.

NEW INSIGHTS ON THE REGULATION OF ENNIATIN PRODUCTION BY *FUSARIUM AVENACEUM* UNDER ABIOTIC CONSTRAINTS

GAUTIER C[1], GALLEGOS N[1], DUMETZ F.[1], ZEHRAOUI E[1], CHEREAU S :[1], RICHARD-FORGET F.[1,*], PONTS N[1]
[1]*Centre de Recherche INRA Bordeaux-Aquitaine, UR1264 MycSA, Villenave d'Ornon, France*
Florence.forget@inrae.fr

The secondary metabolites enniatins are frequently occurring mycotoxins worldwide, mainly produced by the species *Fusarium tricinctum* and *Fusarium avenaceum* on wheat ears. Yet, very little is known regarding the regulatory events controlling their biosynthesis. Among all factors known to greatly influence the production of secondary metabolites in *Fusarium* spp., such as the deoxynivalenol-producing species *Fusarium graminearum*, ambient pH and its fluctuations are of tremendous importance. Here, we explored the potential role of ambient pH at regulating enniatin production by *F. avenaceum*. We found that enniatin production is permitted under a wide range of pH, with apparent optima that depended on the considered strain. Reverse genetics investigations revealed that a putative PacC homologue, herein called FavPac1 for simplicity, may be involved in positively regulating enniatin production at pH 7 and above. Regulations under acidic conditions remain however elusive.

DISCOVERING THE VIROME'S COMPOSITION OF TWO *ASPERGILLUS FLAVUS* POPULATIONS

DEGOLA F.[1*], SPADOLA G.[1], FORGIA M.[2], RESTIVO F.M.[1], TURINA M.[2], CHITARRA W.[2,3], NERVA L.[2,3]

[1]*Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco delle Scienze 11/A, 43125, Parma, Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino, Italy.* [3]*Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano, Italy.*

francesca.degola@unipr.it

The comprehension of the possible biological variables involved in the mycotoxins biosynthesis should include the ecological perspective: in fact, biotic factors have been highlighted as mostly crucial. The multi-level interaction between microorganisms has long-time been proven to be essential for the success of spreading and survival in the environment, by providing adaptive advantages also through the involvement of both molecular and biochemical targets. In fungi, many reports have demonstrated that the presence of viruses could modulate various physiological parameters in some relevant genera, such as the growth rate, the virulence against the host and the biosynthesis of secondary metabolites. Ever since mycoviruses infecting Aspergillus species have been detected, the research efforts have provided evidences that enhanced the understanding of both viral diversity and significance of virus infection/symbiosis in fungi. Recently this aspect shed new light on the phenomena of intraspecific biocompetition in mycotoxigenic species, and, more specifically, on the interplay between aflatoxigenic/atoxigenic strains. This could provide highly effective tools for the improvement of sustainable strategies for the aflatoxins containment in susceptible cereal commodities.

We investigated the composition of the mycovirome characterizing two collections of *Aspergillus flavus* strains, isolated from maize grains sampled in the Northern Italy and in Madagascar; physiotype and chemotype of strains were compared with respect to the presence and the type of viruses, providing, thanks to the very different ecological niches (and thus to a forced adaptation to a different environmental biodiversity) a wider opportunity to clarify some fundamental biological aspects of the most relevant aflatoxigenic specie.

FUNGAL GENOME DIVERSITY AND MYCOTOXIN PRODUCTION

PROCTOR R.H.[1*], MCCORMICK S.P.[1], CARDOZA E.E.[2]. BUSMAN M.[1], KIM H.-S.[1], VAUGHAN M.M.[1], GUTIÉRREZ S.[2]

[1] *USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, Illinois, United States*
[2] *University Group for Research in Engineering and Sustainable Agriculture (GUIIAS), Area of Microbiology, University of León, 24400 Ponferrada, Spain*
robert.proctor@usda.gov

There are over 200 structurally distinct analogs of trichothecene toxins, including the *Fusarium* mycotoxins deoxynivalenol, nivalenol and T-2 toxin. Production of these toxins is conferred by at least 20 trichothecene biosynthetic genes (*TRI* genes), including a gene (*TRI5*) encoding a terpene synthase that converts the primary metabolite farnesyl diphosphate to trichodiene, the parent compound of all trichothecenes. Analysis of *TRI* gene content in species of 12 fungal genera revealed evidence for 31 alterations (acquisitions, losses or changes in function) of the genes that together account for much of the known structural diversity of trichothecenes. The analysis also revealed that some *Trichoderma* species have lost all *TRI* genes except for a functional homolog of *TRI5*. These findings raise the question, what are the causes of such extensive genetic and structural variation? One possibility is that a structural change conferred by acquisition, loss or functional change of a *TRI* gene could allow trichothecene-producing fungi to avoid the effects of substrate-specific trichothecene detoxification enzymes produced by plants and other organisms. A second possibility is evident from heterologous expression of *TRI5* in the biocontrol agent *Trichoderma harzianum*, which normally lacks *TRI* genes. Production of volatiles, including trichodiene, by *TRI5*-expressing *T. harzianum* suppressed *TRI* gene expression and trichothecene production in *F. graminearum*. This suggests that biological activity of trichodiene could drive retention of a functional *TRI5* in *Trichoderma* species that lack other *TRI* genes. Further examination of these possible causes of genetic and structural diversity should provide insight into methods that reduce trichothecene contamination in crops.

GENOMIC COMPARATIVE ANALYSIS AND GENOME EDITING REVEALED THE INVOLVEMENT OF A CYCLASE GENE IN THE BIOSYNTHESIS OF OCHRATOXIN A

FERRARA M.[1], GALLO A.[2], CERVINI C.[3], GAMBACORTA L.[1], SOLFRIZZO M.[1], BAKER S.E.[4,5], PERRONE G.[1]

[1]Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Bari, Italy; [2]Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Lecce, Italy; [3]Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, UK; [4]Functional and Systems Biology Group, Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, WA, United States of America; [5]DOE Joint Bioenergy Institute, Emeryville, CA, United States of America

massimo.ferrara@ispa.cnr.it

The widespread use of Next-Generation Sequencing (NGS) for fungal genome sequencing has led to identification of SM clusters for known metabolites as well as a significant number of novel predicted SM gene clusters. Fungal genome sequencing has great utility for identification of secondary metabolites gene clusters for known as well as novel compounds. Ochratoxin A (OTA) is a well-known mycotoxin with wide distribution on food and feed. A comparative analysis of gene cluster structure in 19 *Aspergillus* and 2 *Penicillium* OTA producers has revealed a high synteny across these species in OTA cluster, encoding five structural genes: *otaA*, *otaB*, *otaC*, *otaR1*, and *otaD*. Furthermore, we identify a previously undescribed additional gene. The new *otaY* gene is located between the *otaA* and *otaB* genes and encodes a protein with predicted SnoaL domain. These snoaL-domain containing proteins have been shown to catalyze ring closure steps in the biosynthesis of polyketide antibiotics produced in *Streptomyces*. Gene expression analysis has demonstrated an up-regulation of the cyclase gene in *A. carbonarius* under OTA permissive conditions, consistent with the expression trends of the other OTA cluster genes. The role of in OTA biosynthesis has been demonstrated by CRISPR/Cas9 complete gene deletion. The presented results reveal for the first time the involvement of a cyclase gene in OTA biosynthetic pathway and redefine the structure of the OTA core cluster, consisting in six genes.

ASPERGILLUS SECTION NIGRI IN ONION BULBS AND PROSPECTION FOR THE INCIDENCE OF GENES INVOLVED IN OCHRATOXIN AND FUMONISIN BIOSYNTHESIS

FUNGARO M.H.P.[1,2]*, MASSI F.P.[1], SARTORI D.[1], TANIWAKI M.H.[2], DA SILVA J.J.[1,2], LIVIERO R.[1], FERRANTI L.[1], IAMANAKA B.T.[2].

[1]*Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil,* [2] *Food Technology Institute, ITAL, Campinas, São Paulo, Brazil.*

mariafungaro@gmail.com.

The aim of this study was to investigate the diversity of *A.* section *Nigri* in onion bulbs acquired monthly in Brazilian retailers and analyze the fungal isolates by means of PCR multiplex to separate the *A. niger* clade species (*A. niger sensu stricto* and *A. welwitschiae*) from other non-toxigenic species of black aspergilli, as well as prospect the presence of genes involved in the biosynthesis of OTA and FB_2. Using a PCR multiplex we detected that almost all isolates (97%) belonged to *A. niger* clade. The *fum8* gene, involved in FB_2 biosynthesis, was found in 36.4% of *A. niger* clade isolates, while on the other hand, the *radH* and *pks* genes, involved in OTA biosynthesis, were found in only 2.8% of these isolates. *A. brasiliensis, A. japonicus, A. tubingensis, A. luchuensis, A. neoniger* and *A. heteromorphus*, which are non-toxigenic species, were isolated from onions but at very low levels. Among *A. niger* clade strains, 242 were subjected to partial sequencing of the *CaM* gene to confirm their identity. Based on the percent identity found in BLASTn and on a phylogenetic inference, 97.9% of the isolates were identified as *A. welwitschiae* and only 2.1% as *A. niger*. The occurrence of OTA and / or FB_2 was also investigated in the onions, however, no contamination was found. In conclusion, among the black aspergillus isolated from onions, there is a moderate and low incidence of fumonigenic and ochratoxigenic strains, respectively, and *A. welwitschiae* was the predominant species in Brazilian onions bulbs.

This research was financially supported by CNPQ (Project 311240/2019-4 and 303732/2018-0) and Fundação de Amparo a Pesquisa do Estado de São Paulo (Project 2019/06032-8)

DIOCTATIN DYSREGULATES MITOCHONDRIAL PROTEASE CLPP AND INDUCES METABOLIC SHIFT TO INHIBIT AFLATOXIN PRODUCTION.

FURUKAWA T.[1*], KATAYAMA H.[2], OIKAWA A. [3,4], NEGISHI L.[5], ICHIKAWA T.[2], SUZUKI M.[6], MURASE K.[6], TAKAYAMA S.[6], SAKUDA S.[1].

[1]Department of Biosciences, Faculty of Science and Engineering, Teikyo University, 1-1 Toyosatodai, Utsunomiya-shi, Tochigi, Japan; [2]Department of Applied Biochemistry, School of Engineering, Tokai University, 4-1-1 Kitakaname, Hiratsuka-shi, Kanagawa, Japan; [3]Department of Food, Life, and Environmental Sciences, Faculty of Agriculture, Yamagata University, 1-4-12 Kojirakawa-machi Yamagata-shi, Yamagata, Japan; [4]RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-chou, Tsurumi-ku, Yokohama-shi, Kanagawa, Japan; [5]Institute for Quantitative Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan; [6]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1Yayoi, Bunkyo-ku, Tokyo, Japan.

furukawa@nasu.bio.teikyo-u.ac.jp

Aflatoxin contamination of crops is a serious problem impacting human health and the economy. Utilization of aflatoxin production inhibitors is attractive, as the elucidation of their modes of action provides clues to clarify the mechanism of aflatoxin production. Dioctatin, a water-soluble analog of *Streptomyces* sp. metabolite dioctatin A, has a potent inhibitory activity against aflatoxin production of *Aspergillus flavus*. Using dioctatin-immobilized affinity beads, we have identified mitochondrial protease ClpP as the binding target of dioctatin. ClpP cannot degrade casein protein without its partner chaperone, ClpX, which unfolds the substrate protein. However, dioctatin enabled recombinant ClpP of *A. flavus* to degrade casein without ClpX. Proteomic analysis showed that dioctatin-bound ClpP selectively degraded mitochondrial energy-related proteins, such as subunits of respiratory complexes and TCA cycle enzymes. Metabolomic analysis revealed that addition of dioctatin increased a glycolysis intermediate and extracellular ethanol production, while reduced several TCA cycle metabolites. Consistently, expression of glycolytic genes and alcohol fermentation genes was upregulated by dioctatin. Moreover, dioctatin decreased acetylation of histones H_3 and H_4, and this might cause the downregulation of expression of aflatoxin cluster genes. These results suggested that abnormal degradation of mitochondrial energy-related proteins by dioctatin-bound ClpP induces metabolic shift to glycolysis and alcohol fermentation, leading to decreases of histone acetylation and aflatoxin production.

This research was financially supported by the Research Project for Improving Food Safety and Animal Health (Ministry of Agriculture, Forestry and Fisheries, Japan, Project number 13406478-4) and the Grant-in-Aid for Early-Career Scientists (Project/Area Number 19K15760).

CHROMOSOME-LEVEL ASPERGILLUS FLAVUS REFERENCE GENOME REVEALS LARGE INSERTIONS POTENTIALLY CONTRIBUTING TO ISOLATE STRESS TOLERANCE AND AFLATOXIN PRODUCTION

FOUNTAIN J.C. [1,2,*], CLEVENGER J.P.[3], NADON B.[4], VAUGHN J.N. [5], YOUNGBLOOD R.C. [5], KORANI W.[4], STARR D.[4], CHANG P.-K. [6], ISETT R.B.[7], JOHNSTON H.R.[7], WIGGIN S .R[4], AGARWAL G[1], KEMERAIT R.C.[1], PANDEY M.K.[8], BHATNAGAR D.[6], OZIAS-AKINS P.[4], VARSHNEY R.K. [8], SCHEFFLER B.E.[5], GUO B.[2,*]

[1] Department of Plant Pathology, University of Georgia, Tifton, GA 31793, USA; [2] USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA 31793, USA; [3] Legume Research, Mars Incorporated, Athens, GA 30605, USA; [4] Institute for Plant Breeding, Genetics, and Genomics, University of Georgia, Athens, GA 30605, USA; [5] USDA-ARS, Genomics and Bioinformatics Research Unit, Stoneville, MS 38776, USA; [6] USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124, USA;
[7] Emory Integrated Genomics Core, Emory University, Atlanta, GA 30322, USA; [8] International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India.
jfount1@uga.edu; baozhu.guo@usda.gov

Previous efforts made in genome sequencing in the *Aspergillus* genus have led to the development of high quality reference genomes for several important species including *A. nidulans*, *A. fumigatus*, and *A. oryzae*. However, less progress had been made with regard to the agriculturally important species *A. flavus*. Efforts began in 2001 to sequence the genome of the isolate NRRL3357 resulting in a scaffold-level genome released in 2005. Since then, the isolate AF70 was sequenced to the scaffold level using an Illumina approach in 2015, and the NRRL3357 isolate was re-sequenced and assembled to chromosome lengths using long-read approaches in 2019. While these resources provide a base for genomics research in *A. flavus*, additional assemblies coupled with comparative and phylogenetic analyses will provide new insights into differences among isolates related to mycotoxin production, pathogenicity, and other phenotypic traits. To explore these important traits and the underlying genetic variation contributing to their diversity, here we present a new, chromosome-level reference genome for AF13, a MAT1-2, highly stress tolerant, and plant pathogenic isolate of *A. flavus*, and a comparative analysis with a complete, chromosome-level assembly of NRRL3357. This analysis resulted in the identification of 153 and 45 unique genes in AF13 and NRRL3357, respectively. Structural variation analyses coupled with optical mapping also confirmed the presence of a large 310Kb insertion present in AF13 containing 58 genes unique to the isolate. Analysis of this insertion revealed the presence of a bZIP transcription factor, *atfC*, which may contribute to isolate pathogenicity and stress tolerance. Additional pathogenicity and stress tolerance-related unique genes were also observed within the insert and elsewhere in the AF13 genome, and may provide an explanation for the greater level of stress tolerance observed in AF13 compared to NRRL3357 and other isolates of *A. flavus*

DE NOVO GENOME ASSEMBLY AND FUNCTIONAL ANNOTATION FOR *FUSARIUM LANGSETHIAE*

ZUO Y.[1], VERHEECKE-VAESSEN C.[2], MOLITOR C.[1], MEDINA A.[2], MAGAN N.[2], MOHAREB F.[1]

[1] *The Bioinformatics Group, Cranfield Soil and Agrifood Institute, School of Water, Energy and Environment, Cranfield University, College Road, Bedford, MK43 0AL, UK.* [2]*Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, College Road, Bedford, MK43 0AL, UK.*

f.mohareb@cranfield.ac.uk

Fusarium langsethiae is a T-2 and HT-2 mycotoxins producing Fusarium species firstly characterised in 2004. It is commonly isolated from oats in Northern Europe. T-2 and HT-2 mycotoxins exhibit immunological and haemotological effects in animal health mainly through inhibition of protein, RNA and DNA synthesis. The development of a high-quality and comprehensively annotated assembly for this species is therefore essential in providing the molecular understanding and the mechanism of T-2 and HT-2 biosynthesis in *F. langsethiae* to help develop effective control strategies. The *F. langsethiae* assembly was produced using PacBio long reads, which were then assembled independently using Canu, SMARTdenovo and Flye; producing a genome assembly total length of 59Mb and N50 of 3.51Mb. A total of 19,336 coding genes were identified using RNA-Seq informed ab-initio gene prediction. Finally, predicting genes were annotated using the basic local alignment search tool (BLAST) against the NCBI non-redundant (NR) genome database and protein hits were annotated using InterProScan. Genes with blast hits were functionally annotated with Gene Ontology.

The newly annotated genome can be downloaded on NCBI and is giving a wonderful opportunity for researchers focusing on *F. langsethiae* to study its transcriptome response to abiotic and biotic factors, including climate change scenarios.

This research was financially supported by a BBSRC-SFI research grant (BB/P001432/1) between Cranfield University, UK and the University College Dublin, Ireland.

CLIMATE CHANGE FACTORS: KINETICS OF GENOMIC AND METABOLOMIC SHIFTS OF *ASPERGILLUS FLAVUS* COLONISATION OF STORED MAIZE

MEDINA, A[*1], GILBERT, M.K.[2],SULYOK, M.[3], VERHECKE-VAESSEN,C.[1], GARCIA-CELA, C.[1], MACK, B.M.[2], BHATNAGAR, D.[2], MAGAN, N.[1]
[1]Applied Mycology Group, Cranfield University, MK43 oAL, UK; [2]USDA, Agricultural Research Service, New Orleans, LA; [3]Centre for Analytical Chemistry, University of Natural Resources and Life Sciences Vienna (BOKU), Austria.
a.medinavaya@cranfield.ac.uk

There is significant interest in the impact that climate change (CC) factors may have on mycotoxigenic fungi. Previously, we examined the impact of three-way interactions between water availability, temperature and elevated CO_2 have on expression of all the genes in the aflatoxin biosynthetic gene cluster using RNAseq, and the impact on aflatoxin B_1 (AFB_1) production by *Aspergillus flavus*. The objectives of this study were to examine the temporal kinetics of colonisation and toxin contamination of stored maize in relation to interacting conditions of temperature (30 *vs* 37°C), CO_2 (400 *vs* 1000 ppm) and drought stress (0.985 *vs* 0.930 water activity) over periods of 8 days storage. Both RNAseq and secondary metabolite production (SMs) changes were quantified. The RNAseq data showed that there were differential impacts on numbers of genes up and down regulated. Under CC conditions, there was a stimulation of certain gene groups, especially those related to stress and abiotic physical factors. Weighted gene co-expression network analyses was used to identify the clusters which changed significantly relative to their role in secondary metabolite and pigment production. The gene networks were examined for relating those clusters that were significantly modified by either elevated CO_2, temperature or water stress shifts.
In terms of secondary metabolite shifts, up to 167 other fungal secondary metabolites in the kinetic study of maize were found after 4 and 8 days storage. Aflatoxin B_1 production increased under elevated CO_2 conditions. Similarly, metabolomic production shifts were observed for a number of secondary metabolites, including AFB_1 derivatives, metabolites from the aflatoxin pathway and some others during colonisation of the maize grain. This study provides in depth new knowledge using RNAseq and metabolomics analyses of the dynamics and impacts of CC scenarios may have on mycotoxin contamination of a staple cereal. Such data sets could be effectively utilised to improve the prediction and potential risk of such mycotoxin contamination of staple food crops. This would be particularly important for improving our understanding of sustainable food production, food safety and the food security agenda.

MANGANESE SUPEROXIDE DISMUTASE IS INVOLVED IN OXIDATIVE STRESS DEFENSE, MITOCHONDRIAL STABILITY AND APOPTOSIS PREVENTION IN FUSARIUM VERTICILLIOIDES

SZABÓ ZS. [1], PÁKOZDI K.[1], MURVAI K.[1], KECSKEMÉTI Á.[2], GÁSPÁR A.[2], OLÁH V.[3], LOGRIECO A.F.[4], DIENES B.[5], CSERNOCH L.[5], ÁDÁM A.L.[6], HORNOK L.[7], PÓCSI I. [1], LEITER É.[1]

[1]Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, H-4032 Debrecen, Egyetem tér 1., Hungary; [2]Department of Botany, Faculty of Science and Technology, University of Debrecen, H-4032 Debrecen, Egyetem tér 1., Hungary;[3]Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, University of Debrecen, H-4032 Debrecen, Egyetem tér 1., Hungary. [4]Institute of Sciences of Food Production (ISPA-CNR), 70126 Bari, Via Amendola 122/O, Italy; [5]Department of Physiology, Faculty of General Medicine, University of Debrecen, H-4012 Debrecen, Nagyerdei krt. 98., Hungary ; [6]Plant Protection Institute, Centre for Agricultural Research, H-1022 Budapest, Herman Ottó út 15., Hungary; [7]Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103 Gödöllő, Páter Károly utca 1., Hungary.
pocsi.istvan@science.unideb.hu

Superoxide dismutases are key enzymes in the elimination of reactive oxygen species (ROS) generated intracellularly or derived from extracellular sources. In this study, we investigated the physiological role of the gene *FvmnSOD* coding for the manganese superoxide dismutase of the maize pathogen *Fusarium verticillioides via* the construction and phenotypic characterization of the gene deletion mutant Δ*FvmnSOD* in comparison to the appropriate wild-type and genetically complemented strains.

Deletion of *FvmnSOD* increased the sensitivity of the fungus to KCl and particularly to menadione on Czapek-Dox stress agar plates. *FvmnSOD* gene deletion also resulted in changes concerning both the morphology and physiology of mitochondria since the volumetric ratio of mitochondria in the second hyphal segment as well as the total, the KCN-sensitive cytochrome c-dependent and the KCN+SHAM resistant residual respiration rates were higher in the mutant compared to the wild-type strain. Changes in the mitochondrial integrity brought about higher sensitivity to the *Penicillium chrysogenum* antifungal protein-elicited apoptotic cell death, similarly to the Δ*mnSOD* mutant of *A. nidulans*. The elimination of *FvmnSOD* led to decreased fumonisin B1 toxin production when analysed in Myro medium but did not disturb either the invasive growth - tested on unwounded tomato fruits - or the fertility of the fungus.

The research was financed by the European Union and the European Social Fund through the project EFOP-3.6.1–16–2016-00022, by the National Research, Development and Innovation Office (Hungary) K112181 and K119494 grants and by the Higher Education Institutional Excellence Program (NKFIH-1150-6/2019) of the Ministry of Innovation and Technology in Hungary, within the framework of the Biotechnology thematic program of the University of Debrecen.

IN VITRO EFFICACY OF PROTHIOCONAZOLE, DIFENOCONAZOLE AND THEIR MIXTURE AGAINST TOXIGENIC *FUSARIUM* SPECIES PATHOGENS ON CEREALS

SOMMA S.[1], MASIELLO M.[1*], POLLMANN B.[2], HAIDUKOWSKI M.[1], LOGRIECO A.F.[1], MORETTI A.[1]

[1]*National Research Council, Institute of Sciences of Food Production, Bari, Italy;* [2]*ADAMA MAKHTESHIM LTD, Israel*

mario.masiello@ispa.cnr.it

Fusarium species are common fungi of cereals representing a serious concern for both their pathogenicity on plants and the ability to synthetize harmful mycotoxins. The most important disease on wheat is Fusarium Head Blight caused by the co-occurrence of several *Fusarium* species. In the recent years, the growing willingness to reduce the impact of chemicals in agriculture triggered many investigations aimed to make more effective fungicides doses, in order to limit wasteful treatments and at the selection of fungal strains less sensitive at sub lethal doses. The effectiveness of two DMI fungicides, prothioconazole and difenoconazole and their mixture has been tested on the most common toxigenic *Fusarium* species occurring on cereals: *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, *F. proliferatum* and *F. langsethiae*. The efficacy of six increasing fungicide doses (from 0.1 to 100 mg L^{-1}) was evaluated by mycelial growth inhibition test, considering 3 strains for each *Fusarium* species. Prothioconazole was proved to be the most effective, since all strains were completely inhibited at 10 mg L^{-1}, up to 10 days of incubation. On the contrary, after 10 days of incubation, mycelial growth was not completely arrested at the highest concentration of difenoconazole (CMI > 100 mg L^{-1}). In addition, a different response among and within *Fusarium* tested species was observed. Mycelial growth was inhibited of 50% already at low doses, up to 5 mg L^{-1}, of mixture and where completely arrested at the highest doses tested. Furthermore, preliminary analyses showed that fungicide doses did not influence *in vitro* mycotoxin production.

REDUCTION OF DON CONTAMINATION IN WHEAT GRAINS PRODUCED IN HUMID SUBTROPICAL AREAS OF PARANÁ STATE, SOUTHERN BRAZIL, WITH SEQUENTIAL FUNGICIDE APPLICATIONS

FEKSA H.R. [1], MOURÃO M.G.[1], GARDIANO C.G.[1], DUHATSCHEK B.[1], SANTOS P.C.[1] , TESSMANN D.J.[2].

[1]*Fundação Agrária de Pesquisa Agropecuária/Cooperativa Agraria Agroindustrial, Distrito de Entre-Rios, CEP 85139-400, Guarapuava, Paraná, Brazil;* [2]*Departamento de Agronomia, Universidade Estadual de Maringá, CEP 87020-900, Maringá, Paraná, Brazil.*

djtessmann@uem.br

Wheat production in the humid subtropical areas of Paraná State, in southern Brazil, is frequently affected by Fusarium head blight (FHB) epidemics caused by the *Fusarium graminearum* species complex. High humidity during the wheat production cycle and a no-till farming system, with two crops per year, increase the inoculum of pathogens. The perception of the contamination risk by DON has increased in Brazil since the maximum limits for unprocessed grains (3000 µg/kg) and flour (750 µg/kg) were regulated. The market has required even lower levels than these. As fungicides are essential for FHB and DON management, we evaluated the efficacy of sequential fungicide applications in the control of FHB and DON. Experiments were conducted under natural infection with the soft wheat cultivar Campeiro from 2014 to 2017, comparing one, two, and three fungicide sprays after mid-flowering at seven-day intervals. An untreated check was also included. Tebuconazole was used in the first and third sprays, and Carbendazim was used in the second spray. The experimental design was a randomized complete block with six replications, and treatments were in a factorial arrangement. There was a significant effect of year, number of applications, and interaction between these two factors (P≤0.05) on FHB and DON. DON accumulation in grains of treatments having one, two, and three fungicide sprays and the untreated check, ranged from 2109–3725 µg/kg, 469–1311 µg/kg, 198 –933 µg/kg, and 4659–7203 µg/kg, respectively. These results have been useful for the management of FHB and DON in that region.

This research was financially supported by the Agraria Foundation for Agricultural Research, Agraria Agroindustrial Cooperative and National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico, project 310719/2016-6) of Brazil.

VARIATION IN SECONDARY METABOLITE PRODUCTION POTENTIAL IN THE FUSARIUM INCARNATUM-EQUISETI SPECIES COMPLEX REVEALED BY COMPARATIVE ANALYSIS OF 13 GENOMES

VILLANI A.[1], PROCTOR R.H.[2], KIM H.-S. [2], BROWN D.W. [2], LOGRIECO A.F.[1], AMATULLI M.T.[1,3], MORETTI A.[1], SUSCA A.[1]

[1]Institute of Sciences of Food Production, National Research Council, Bari, Italy; [2]Department of Agriculture Peoria, National Center for Agricultural Utilization Research, U.S., Peoria, IL, USA; [3]Thales Alenia Space Italia, Torino, Italy.

antonio.moretti@ispa.cnr.it

Fusarium incarnatum-equiseti species complex (FIESC) comprises 33 phylogenetically distinct species recovered from diverse biological sources, but mostly agricultural plants and soils. Collectively, members of FIESC can produce diverse mycotoxins. However, since such species diversity in FIESC has been recognized recently, each species ability of causing mycotoxin contamination of crop plants is unclear. We used comparative genomics to investigate the distribution of and variation in genes and gene clusters responsible for the synthesis of mycotoxins and other secondary metabolites (SMs) in FIESC.

We examined genomes of 13 members of FIESC, selected based primarily on their phylogenetic diversity and/or occurrence on crops. Presence and absence of SM biosynthetic gene clusters varied markedly among the genomes. For example, trichothecene mycotoxin as well as the carotenoid and fusarubin pigment clusters were present in all genomes examined, whereas enniatin, fusarin, and zearalenone mycotoxin clusters were present in only some genomes. Some clusters exhibited discontinuous patterns of distribution in that their presence and absence was not correlated with the phylogenetic relationships of species. We also found evidence that cluster loss and horizontal gene transfer have contributed to such distribution patterns. For example, a combination of multiple phylogenetic analyses suggest that five NRPS and seven PKS genes were introduced into FIESC from other Fusarium lineages. Our results suggest that although the portion of the genome devoted to SM biosynthesis has remained similar during the evolutionary diversification of FIESC, the ability to produce SMs could be affected by the different distribution of related functional and complete gene clusters.

to Index

Session S6

Control of mycotoxigenic fungi and mycotoxins in the field

REDUCING AFLATOXIN CONTAMINATION IN SEVERAL AFRICAN NATIONS THROUGH USE OF COMMERCIAL ATOXIGENIC BIOLOGICAL CONTROL PRODUCTS

BANDYOPADHYAY R.[1], ORTEGA-BELTRAN A.[1]*, AKANDE A.[2], KONLAMBIGUE M.[3]
[1]International Institute of Tropical Agriculture (IITA), Ibadan 200001, Nigeria; [2]IITA, Abuja, Nigeria. [3]IITA, Accra, Ghana.
a.beltran@cgiar.org

Aflatoxins pose a significant public health risk, decrease productivity and profitability of animal industries, and hamper trade. To minimize aflatoxin contamination in several crops, a biocontrol technology based on atoxigenic strains of *Aspergillus flavus* that do not produce aflatoxin is used widely in the US. The technology has been improved and adapted for use in Africa by the International Institute of Tropical Agriculture (IITA) in partnership with several national and international institutions under the tradename Aflasafe. Country-specific Aflasafe products have been developed or are currently being developed in 20 African nations. The four atoxigenic *A. flavus* genotypes used as active ingredient fungi in the biocontrol formulations competitively displace aflatoxin producers during crop development and this results in less aflatoxin content. Using incentivization mechanisms (AgResults: https://agresults.org/projects/nigeria) and commercialization approaches (www.aflasafe.com), Aflasafe is being scaled up through a mix of public, private, and public-private partnership interventions. The use of biocontrol is accompanied by practical pre- and post-harvest technologies, awareness and sensitization campaigns, testing, market development, dietary and policy interventions, among other strategies/technologies. The holistic interventions are designed in coordination with all relevant stakeholders across value chains of each target nation. Implementing tailored management strategies allows consistent production of aflatoxin-compliant crops in an affordable, economic manner. Crops produced using the tailored interventions typically accumulate over 80%, sometimes 100%, less aflatoxins than untreated crops grown, processed, and stored using traditional practices. Several African nations have commercially treated more than 300,000 ha of maize and groundnut crops achieving >90% less aflatoxins. We recommend use of biocontrol as one of the main components of integrated management of aflatoxins. This presentation will provide information on the long road from development to large-scale deployment of biological control for improving food security, promoting trade, contributing to healthier farm families, and creating wealth.

BIOCONTROL ABILITY OF THE *METSCHNIKOWIA* SPP. AGAINST *ASPERGILLUS FLAVUS* AND ITS AFB1 PRODUCTION

DIKMETAS D. N.[1], OZER H.[2], KARBANCIOGLU-GULER F.[1]
[1]*Istanbul Technical University, Department of Food Engineering/ Faculty and Mettalurgical Engineering, Istanbul, Turkey.* [2]*Tubitak MAM Food Institue/Tubitak MAM, Kocaeli, Turkey.*
dikmetas@itu.edu.tr

Aflatoxins which are carcinogenic mycotoxins produced by particularly *Aspergillus flavus*, and are known to become a global concern in the agricultural market, the most potent one is the aflatoxin B_1. This study was designed to examine the biocontrol potential of five *Metschnikowia* spp. (1-UDM, 26-BMD, 32-AMM, DN-MP, DN-UY), isolated from different parts of plants collected from Turkey against the AFB_1 producer *A. flavus* and their capability to reduce AFB_1 production with *in vitro* studies. Fungal growth was evaluated with radial inhibition assay by measuring of colony area on potato dextrose agar (PDA) at 25 ºC. Moreover, aflatoxin B_1 production properties were examined after 3, 5 and 7 days of incubation by high performance liquid chromatography. Yeast cultures were significantly effective to inhibit *A. flavus* growth especially in, two yeast cultures, 1-UDM and 32-AMM. According to our results, antifungal activity of yeast cultures influenced from the incubation time ($p<0.05$). After 7 days of incubation, yeast treated samples showed more than 90% reduction in all yeast isolates, at 3, 5 and 7 days of incubation. This study suggests that, application of antagonistic yeast may be an alternative approach to control *A. flavus growth* and its AFB_1 production with more safe, feasible and green strategy.

This research was financially supported by the the Scientific Research Council of Istanbul Technical University (grant no: MYL- 2020-42498).

THE MECHANISM OF FORMATION OF LACTYL- AND PROPIONYL-DON

MICHLMAYR H.[1], SOPEL M.[2], HAMETNER C.[3], TWARUSCHEK K.[1], WIESENBERGER G.[1], WÖLFLINGSEDER L.[4], MARKO D.[4], BERTHILLER F.[1], ADAM G.[1].

[1]*Institute of Microbial Genetics, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna (BOKU), 3430 Tulln, Austria.* [2]*Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU), 3430 Tulln, Austria.* [3]*Institute of Applied Synthetic* Chemistry, *TU Wien, Austria.* [4]*Department of Food Chemistry and Toxicology, University of Vienna, Austria.*
gerhard.adam@boku.ac.at

The use of lactic acid bacteria is increasingly proposed for stabilization *of Fusarium* damaged and deoxynivalenol (DON) contaminated grain. The compound 3-lactyl-DON was reported in 1982 as the toxic principle "responsible for vomiting in humans and swine" in naturally contaminated barley in China. Theoretically, 3-lactyl-DON could be produced by a) DON challenged lactic acid bacteria, b) by plant cells (in the presence of DON and lactic acid), or c) by lactic acid stressed *Fusarium*. We found that the FgTri101 acetyltransferase can utilize lactyl-CoA instead of acetyl-CoA to acylate the C3-OH of DON. Lactate is utilized via lactate dehydrogenase and pyruvate-dehydrogenase, but normally no lactyl-CoA is formed. Yet, *Escherichia coli* posesses *prpE* encoding propionyl-CoA synthase that can also act as lactyl-CoA synthase (ATP + CoA + Lactate = Lactyl-CoA + AMP + PPi). Combining recombinant prpE and FgTri101 proteins *in vitro* with these substrates allowed enzymatic synthesis of both 3-lactyl-DON and 3-propionyl-DON in good yield, which were purified by preparative HPLC. The structures were confirmed by NMR. *F. graminearum* possesses a functional propionyl-CoA synthase (FGSG_10126) which can fully replace prpE. Interestingly, both lactyl-DON and propionyl-DON can be hydrolyzed back to DON by the 15-ADON chemotype Tri8 esterase, and also by human cells. The enzymatically generated standards are currently used to investigate under which conditions the "new" (potentially re-emerging) modified mycotoxins are formed, and to characterize their toxicological properties.

Funded by the Austrian Science Fund FWF (SFB Fusarium F3702, F3715 and F3718).

FROM FIELD TO ISOLATE, A NOVEL METHOD FOR SELECTING EAR COLONIZING BACTERIA TO CONTROL FUSARIUM GRAMINEARUM IN WHEAT

DEROO W.[1,2,3*], VANDENABEELE S.[1], DE GELDER L.[2], AUDENAERT K.[3]
[1]Aphea.bio, Ghent, Belgium; [2]Laboratory of Environmental Biotechnology, Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University; [3]Laboratory of Applied Mycology and Phenomics, Department of Plants and Crops, Ghent University, Ghent, Belgium.
waldo.deroo@ugent.be

Global pressure on the continued large-scale use of chemical fungicides has made way for the era of agro-bioloicals. Immense potential lies in the discovery and validation of a potent and resilient biocontrol organisms to combat agricultural pests. Yet, the implementation is hampered by fluctuant efficiency due to the poor survival of these organisms in an agro-ecosystem. *Fusarium graminearum (Fg)*, the most potent species in the *Fusarium* head blight disease complex in wheat is devastating to cereal crops worldwide. It decreases the grain yield and causes mycotoxin contamination, rendering the harvest worthless at heavy infestations. In this study whole ear microbiomes were extracted from 3 different wheat (*Triticum eastivum*) and 1 spelt (*Triticum spelta*) cultivars in the field. These microbiomes were inoculated on wheat spikes in a detached spike assay through spray application, followed by Fg spray inoculation. This infection cycle was repeated 4 times, in parallel lines for the 4 unique starting points. Every time selecting, isolating and re-applying the microbiome from the least infected spikelets from previous cycles onto the next. After 4 cycles, 94 single cell isolates were obtained of the best preforming line. All 94 isolates were tested as biocontrol agent against Fg in a detached leaf infection assay. This novel method provided several potential candidates for in planta control of Fg.

CROPPING FACTORS: THE KEY FOR SUSTAINABLE MYCOTOXIN MANAGEMENT IN CEREALS

SUSANNE VOGELGSANG
Research group Extension Arable Crops, Competence Division Plants and Plant Products, Agroscope, Zurich, Switzerland
susanne.vogelgsang@agroscope.admin.ch

Over an eight-year period, 686 wheat grain samples and information on their cropping history were obtained from Swiss growers. To estimate the risk of Fusarium head blight (FHB), grains were examined for *Fusarium* species incidence, mycotoxin content and the abundance of *F. graminearum* (FG) and *F. poae* (FP) DNA. Of all *Fusarium* species, FG and FP were predominant. Deoxynivalenol (DON), zearalenone (ZEN) and nivalenol (NIV) were the most frequently detected toxins. For DON, 11% and for ZEN, 7% of all samples exceeded the European maximum limits for unprocessed cereals. A multiple correspondence analysis revealed that high levels of FG and DON were mainly observed in grain samples from fields with previous crop maize, reduced tillage, cultivars with poor FG-resistance and strobilurin-based fungicides. Other previous crops and/or ploughing decreased the DON content by 78 to 95%. ZEN showed a similar pattern. In contrast, high levels of FP and NIV were associated with samples from ploughed fields and the previous crop canola. These findings suggest a different ecological niche for FP. In a two-year barley survey (253/237 grain/straw samples), FG and F. avenaceum (FA) were dominant while antibiotic Y (AY; average in grain 105µg kg^{-1} and in straw 188 µg kg^{-1}) showed higher concentrations than DON (122/199 µg kg^{-1}). Similar to wheat, high levels of FG and DON in barley were associated with previous crop maize although tillage showed no effect. In contrary, high abundance of FA and AY were linked to samples from fields with wheat as previous crop.

In order to reduce *Fusarium* mycotoxins in wheat in a maize-wheat rotation under minimum tillage, the effects of cut-and-carry biofumigation and cover cropping systems were investigated under field conditions. Cut-and-carry biofumigation treatments with mulch layers from white mustard, Indian mustard or berseem clover reduced DON content by up to 58% compared with the control treatment. The use of white mustard, Indian mustard or winter pea as interval cover crops after silage maize also reduced DON and improved yield in spring wheat by up to 85% and 25%, respectively. Remarkably, the toxin reduction through these cover crops was comparable with that obtained by ploughing. The outcome of these studies suggest that, within the context of sustainable crop protection strategies, cereal growers and consumers could make use of the recommended prevention measures to improve both grain yield and quality.

References

- Dimitrios D et al. (2020) Prevention of Fusarium head blight infection and mycotoxins in wheat with cut-and-carry biofumigation and botanicals. *Field Crops Research* 246: 107681; https://doi.org/10.1016/j.fcr.2019.107681
- Drakopoulos, D et al. (2021) The agronomic and economic viability of innovative cropping systems to reduce Fusarium head blight and related mycotoxins in wheat. *Agricultural Systems* 192: 103198; https://doi.org/10.1016/j.agsy.2021.103198
- Vogelgsang S et al. (2017) *Fusarium* mycotoxins in Swiss wheat: a survey of growers' samples between 2007 and 2014 shows strong year and minor geographic effects. *Toxins* 2017, 9(8), 246; https://doi.org/10.3390/toxins9080246
- Vogelgsang S et al. (2019) An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins. *European Journal of Agronomy*, 105, 62-77; https://doi.org/10.1016/j.eja.2019.01.002

USE OF FERMENTATION METABOLITES OF LACTIC ACID BACTERIA TO CONTROL MYCOTOXIGENIC FUNGI

CORRÊA J. A. F.[1*], EVANGELISTA A. G.[1], BIAUKI G. C.[2], DA ROCHA G. F.[2], LUCIANO F. B.[1]

[1]*Graduate Program in Animal Science, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, 80215-901 Curitiba, Brazil.* [2]*Undergradute Program in Biotechnology, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, 80215-901 Curitiba, Brazil.*

jessica.feijo@pucpr.edu.br

The contamination of feed, forages and silages by mycotoxins is of major concern in animal production. Lactic acid bacteria (LAB) are potential biocontrol agents for fungal control. Thereon, we evaluated the *in vitro* inhibitory activity of LAB fermentation products (FPs) on fungal growth. FPs were obtained from 17 LAB strains singly cultivated in De Man, Rogosa and Sharpe broth (48 h, 37 °C). Cultures were then centrifuged (5,000×*g*, 10 min) and syringe-filtered (0.22 µm). The minimum inhibitory and fungicidal concentrations (MIC, MFC) were determined for FPs against the mycotoxigenic fungi *Aspergillus sclerotioniger* CECT 20583, *Aspergillus ochraceus* CMT 435, *Aspergillus parasiticus* CECT 2681, *Aspergillus parasiticus* CMT 336, *Fusarium graminearum* CECT 2150, and *Fusarium verticillioides* CECT 2983. Also, four variations of the FPs (unaltered, boiled, neutralized, and previously autoclaved) were assessed. Similar effects were observed when comparing the different producing LAB. Among variations, the neutralized has proven to be the least effective (pH-dependent activity), with higher MIC/MFC when compared to the others. Likewise, the autoclaved variation was the most effective, which may be explained by the liberation of LAB intracellular content. Among fungi, the most resistant species was *A. sclerotioniger* CECT 20583. In general, *Aspergillus* strains have shown higher MIC compared to *Fusarium*. MFC were only achieved for *F. verticillioides* CECT 2983, which endorses the potential application of FPs to control this fumonisin-producing species. Further assays shall be carried out to identify compounds contained in the FPs to elucidate the mechanisms of action involved in the inhibitory effects here described.

This research was financially supported by the Brazilian National Council for Scientific and Technological Development, processes CNPq 437728/2018-8, 142196/2019-3, and 308598/2020-2; by the Coordination for the Improvement of Higher Education Personnel, processes CAPES 001, 88881.623467/2021-01, and 88887.512219/2020-00; and by the Pontifical Catholic University of Parana.

ACTIVITY OF BIOPROTECTIVE LACTIC ACID BACTERIA AGAINST MYCOTOXIGENIC FUNGI

CORRÊA J. A. F.[1*], EVANGELISTA A. G.[1], DA ROCHA G. F.[2], , BIAUKI G. C.[2], LUCIANO F. B.[1]

[1]Graduate Program in Animal Science, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, 80215-901 Curitiba, Brazil. [2]Undergradute Program in Biotechnology, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, 80215-901 Curitiba, Brazil.

jessica.feijo@pucpr.edu.br

Lactic acid bacteria (LAB) have shown antifungal and detoxifying effects towards mycotoxins, prospecting their use for biocontrol. The objective of this work was to evaluate the inhibitory activity of 17 potentially bioprotective LAB strains towards the development of mycotoxigenic strains, aiming at the future application as silage inoculants or probiotics in feed with protective effects against fungal contaminants. For evaluation, fresh cultures of LAB in De Man, Rogosa and Sharpe (MRS) broth were applied at 4 points (6 µL each) equidistantly on MRS agar plates and incubated (37 °C, 24 h) to develop circular spots. Thereafter, plates received 7 ml of pre-solidified soft agar (70%, 45 °C) potato dextrose agar inoculated with the fungus (10^4 spores/mL). The fungal strains assessed were *Aspergillus parasiticus* CECT 2681, *Aspergillus ochraceus* CMT 435, *Fusarium verticillioides* CECT 2983 and *Fusarium graminearum* CECT 2150. Once solidified, the plates underwent a new incubation (25 °C, 72 h) and visual evaluations were performed to measure inhibition halos. Measurements were analyzed according to the following inhibition scale: absence (0 – 4 mm); low (5 – 9 mm); moderate (10 – 14 mm); and high (≥15 mm). Except against *A. parasiticus* CECT 2681, all LAB strains promoted some level of inhibition (areas ≥5 mm). Also, both *Fusarium* strains have proven to be highly susceptible to LAB presence. The use of LAB for fungal control is a promising approach, since it can avoid mold growth and, consequently, mycotoxin production. Further assays shall be carried to also evaluate the detoxifying activity of these bacterial strains.

This research was financially supported by the Brazilian National Council for Scientific and Technological Development, processes CNPq 437728/2018-8, 142196/2019-3, and 308598/2020-2; by the Coordination for the Improvement of Higher Education Personnel, processes CAPES 001, 88881.623467/2021-01, and 88887.512219/2020-00; and by the Pontifical Catholic University of Parana.

INTEGRATED STRATEGIES FOR CONTROLLING FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL CONTAMINATION IN WHEAT

MA H.X.[1], ZHANG X.[1], CHEN H.G. [2], YANG R.M.[3].
[1]*Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, Jiangsu, China;* [2]*Institure of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, Jiangsu, China;* [3]*Jiangsu Station of Plant Protection and Plant Quarantine, Nanjing, 210036, Jiangsu, China.*
hxma@jaas.ac.cn

Fusarium head blight (FHB) disease and deoxynivalenol (DON) contamination of wheat depend on multiple factors. In the study, varieties, sowing time and rate, rotation crop, nitrogen application, fungicide etc. were evaluated for controlling FHB and DON accumulation. 71 wheat varieties were selected for FHB and DON test. 34 varieties from the reaches of Yangtze River showed moderate resistance or resistance to FHB with DON of 1.21~11.5 mg/kg in inoculated grains. 37 varieties from the reaches of Huang and Huai River had less resistance to FHB with DON of 6.61~80.92 mg/kg. Nine varieties of them had moderate resistance to FHB, whereas all others were susceptible. FHB increased with the delay of sowing time and increase of sowing rate. FHB severity was also affected by the crop rotation and nitrogen application. In comparing with no fungicide application, the control effects of pydiflumetofen, prothioconazole, phenamacril, metconazole, tebuconazole and carbendazim were 91.2%, 90.0%, 81.9%, 78.0%, 74.2% and 71% for the proportion of scabbed spikes, and 91.2%, 91.8%, 83.6, 79.4%, 74% and 63.3% for disease index, respectively. The pydiflumetofen, prothioconazole, phenamacril, metconazole, tebuconazole and carbendazim reduced the DON content by 87.0%, 87.0%, 69.6%, 64.1%, 37.9%, and 67.0%, respectively. The fungicide used with mist sprayer in the period of full head and blossom had the best control effect. 15 demonstration areas with more than 50 ha for each were established based on multiple strategies, the control effects of the proportion scabbed spikes and disease index were 83.7% and 86.2% in average, and DON was not detected.

This work was partially supported by the National Key Project for the Research and Development of China (2016YFE0112900, 2016YFD0100500), China Agricultural Research System Program (CARS-03), and European Union Horizon 2020 Mycokey project (EU678781).

STUDIES ON THE EFFICACY OF ELECTROLYSED OXIDISING WATER TO CONTROL ASPERGILLUS CARBONARIUS AND OCHRATOXIN A CONTAMINATION ON GRAPE

MAGISTÀ D.[1*], COZZI G.[1], GAMBACORTA L.[1], LOGRIECO A.F.[1], SOLFRIZZO M.[1] ,PERRONE G.[1]
[1]*National Research Council, Institute of Sciences of Food Production, Bari, Italy.*
donato.magista@ispa.cnr.it

Ochratoxin A (OTA) occurrence in grapes is caused by black Aspergilli (*Aspergillus carbonarius* followed by *A. niger*) vineyards contamination. It depends on climatic conditions, geographical regions, damage by insects, and grape varieties. Good agricultural practices, pesticides, and fungicides seem adequate to manage the problem during low OTA risk vintages, but the development of new strategies is always encouraged, especially when an extremely favourable condition occurs in the vineyard. Electrolysed oxidising water (EOW) has become an interesting alternative to chemicals in agriculture, mainly during the post-harvest phase. This study tested the fungicidal efficacy of EOW generated by potassium chloride, in vitro, on black Aspergilli conidia, and detached grape berries infected by *A. carbonarius*. Then, during field trials on Primitivo cv vineyard treated with EOW, *A. carbonarius* contamination, and OTA levels were compared with Switch® fungicide treatment (0.8 g/l). Black Aspergilli conidia were killed on plate assay after 2 min of treatment by EOW containing >0.4 g/l of active chlorine. EOW (0.6 g/l active chlorine) treatment reduced the rate of *A. carbonarius* infections in vitro of about 87–92% on detached berries and, more than half in the field trials, although Switch® showed better performance. A significant reduction in the OTA concentration was observed for the EOW and Switch® treatments in vitro (92% and 96%, respectively), while in the field trials, although the average decrease in OTA was recorded in the treated grapes, it was not statistically significant. These results highlighted that EOW could be considered effective, as a substitute for fungicides, to reduce the contamination of *A. carbonarius* and OTA on grapes.

Int J Food Microbiol. 2021 Jan 2;338:108996. doi: 10.1016/j.ijfoodmicro.2020.108996
The present work has received funding by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey).

AFLATOX® PROJECT: A BIOTECHNOLOGICAL APPROACH FOR THE DEVELOPMENT OF NEW ANTIFUNGAL COMPOUNDS TO PROTECT THE ENVIRONMENT AND THE HUMAN HEALTH

MONTALBANO S.[1]*, BARTOLI J.[1], BISCEGLIE F.[1], BUSCHINI A.[1], CARCELLI M.[1], CERETTI E.[2], DEGOLA F.[1], FERETTI D.[2], PELOSI G.[1], PIOLI M.[1], RESTIVO F.M.[1], ROGOLINO D.[1], SPADOLA G.[1], ZERBINI I.[2], ZANI [2].

[1]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, 43124 Parma, Italy; [2]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, 25121 Brescia, Italy.

serena.montalbano@studenti.unipr.it

The Aflatox® Project aims to design and develop new bioinorganic inhibitors of aflatoxigenic *Aspergillus spp.* proliferation and toxin production, harmless to environment and human health. Controlling fungal contamination on crops is considered a priority by sanitary Authorities of an increasing number of Countries, due also to the fact that the geographic areas interested in mycotoxin outbreaks are widening. Among the different pre- and post-harvest strategies that may be applied to prevent fungal and/or mycotoxin contamination, fungicides still play a prominent role.

The Aflatox® Project experimental flow-chart provides for a multidisciplinary approach starting from the synthesis of a panel of bioactive molecules derived from natural aldehydes and ketones. We functionalized these compounds in order to modulate their properties, such as solubility, complexing ability, and lipophilicity. Endogenous metal ions were used to obtain the relative metal complexes. We set up a method to determine their effects on fungal germination and growth and aflatoxin biosynthesis. The new compounds were tested to detect mycelia growth inhibition and mycotoxin production against different strains of *A. flavus*. Once the chemical-physical properties and the molecular structures of the new complexes have been elucidated and their antifungal and antimycotoxigenic activity determined, we used different in vitro and in vivo models to evaluate toxicity and genotoxicity of the new compounds and to determine the potential risk for environment and human health with the purpose to use them in the field.

All the data were organized in a Quantitative Structure–Activity Relationship (QSAR) database correlating chemical structures with biological/toxicological activities.

Financial support: Fondazione Cariplo-Project N. 2014-0555, http://aflatox.unibs.it/

MANAGING MYCOTOXINS IN AFRICA AND ROLE OF THE AFRICAN SOCIETY FOR MYCOTOXICOLOGY

OKOTH S.[1], ROSE L.J.[2], FLETT B.C.[3], VILJOEN[2] A.
[1]*School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya.*
[2]*Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.* [3]*Agricultural Research Council, Grain Crops, Potchefstroom, South Africa*
sheilaokoth@uonbi.ac.ke

The associated health burden of mycotoxins and effect on agriculture and trade, is highly visible in Sub-Saharan Africa (SSA). Crops that are most susceptible to mycotoxin accumulation include staple foods like maize, wheat, groundnuts, sorghum, millet and tubers. These are reported to be heavily contaminated with several mycotoxins produced by diverse fungi. Of particular economic and toxicological importance are aflatoxins and fumonisins. Levels of aflatoxin contamination of food and feed as high as 48,000 µg/kg in maize, is reported. The complexity of the contamination sources at pre-harvest and post-harvest levels complicates solutions to mitigation. Members of *Aspergillus* section *Flavi*, where most aflatoxigenic fungi are grouped, require polyphasic approach in their identification and this fact is important in their use as bio-controls and for disease management. Several other aflatoxin mitigation strategies are reported including identification of resistant maize germplasm to Aspergillus and Fusarium ear rots and improved storage structures. Uptake and impact of these technologies, at a scale, requires evaluation. Mitigating aflatoxin and other mycotoxin contaminations require new ways of working together, linking the academic to the technology world. The African Society of Mycotoxicology was formed in 2015 with a vision to promote and collate mycotoxin research carried out by African researchers and their international collaborators to improve synergy and solve the mycotoxin issues in Africa using practical solutions. The society brings together stakeholders, including industry, other private sector and governments, in discussions on managing the African mycotoxin threat.

This research was financially supported by CIMMYT, Finnish Foreign Affairs, National Research Foundation, South Africa, and National Research Fund, Kenya.

.

MYCOTOXINS IN LATIN AMERICA: A BRIEF OVERVIEW OF THE CURRENT SITUATION

TORRES A.
Research Institute on Mycology and Mycotoxicology (IMICO), *National Scientific and Technical Research Council* (CONICET)-*National University of Río Cuarto- (UNRC),* (5800) *Río Cuarto-Córdoba, Argentina.*
atorres@exa.unrc.edu.ar

Latin America is a very heterogeneous region; that differs significantly in several ways, including agriculture; with a wide variety of agro-ecological zones, varied topography and different farm sizes and structures. The agriculture in the Southern Cone is dominated by large-scale and export-oriented farms, particularly in Argentina and Brazil, while much of the rest of the region is characterized by smallholder and family agriculture. From a Latin American context, the major mycotoxins of significance in terms of health and the economy are the aflatoxins, ochratoxin A, fumonisins, zearalenone,and deoxynivalenol. Although the natural occurrence of mycotoxins is relevant in many Latin American countries, relatively few studies have been conducted on dietary mycotoxin intake in the region compared to other regions worldwide, mainly due to lack of advanced laboratory equipment, capacity and expertise, insufficient research funds and limited surveillance systems. The mentioned heterogeneity also can be seen among countries in different issues related with mycotoxins such as control policies, regulations, surveillances, population exposure evaluations; and obviously in the funds allocated for scientific research in this area. Limited information on dietary mycotoxins exists outside of Argentina, Brazil, Chile and Mexico, while monitoring and enforcement of regulatory standards are rare or non-existent. The integration of the Latin American internal market as regards agriculture and food makes it necessary to address the problems that arise in this area, specifically on mycotoxins, and hence to carry out related research, on a Latin American scale. This presentation will give insights into the epidemiology of mycotoxins in agricultural food commodities and discusses recent changes in legislation. Also, it will give a brief overview of studies on population exposure to mycotoxins in different countries, and future challenges on mycotoxin research in Latin America.

.

to Index

Session

12 November 2021

to Index

Session S7

Modelling and ICT solutions

PAST, PRESENT, AND FUTURE OF MODELLING TO PREDICT MYCOTOXIN CONTAMINATION

BATTILANI P.* , CAMARDO LEGGIERI M.
Università Cattolica Sacro Cuore, Dept Sustainable Crop Production, Via E Parmense 84, 29122 Piacenza, Italy.
paola.battilani@unicatt.it

During the crop growth in the field weather parameters are the driving variables for fungal communities. Therefore, predictive models, using meteorological data as main input, were developed focusing on cereals as host crops; the "one-fungus one-crop" approach was pursued and deoxynivalenol in wheat, aflatoxins and fumonisins in maize deserved the major attention. Most of these models were developed following empiric approaches, intended as mathematical functions obtained linking weather data with mycotoxin occurrence data, while few mechanistic models, those including the infection cycle of the fungi, are available. During the last 10-15 years, research interest regarded the geographic and crop extension of predictive models. Mechanistic models confirmed their expected flexibility in the few applications managed.
"Mycotoxin mitigation" as research topic, advanced significantly since 2000. The role of the crop chain management, showed increasing impact and requested its enclosure in predictive modelling. The aid of machine learning techniques is at the beginning of its application to support predictive models, but it is very promising. Further, the impact of climate change undermined the consolidate knowledge on global fungi distribution, while it stressed the emerging issue of fungi co-occurrence. Modelling poorly contributed till now, but it should expand the geographic areas of application and switch to one crop-multi fungi, to support the emerging issue of mycotoxin co-occurrence. In perspective, other tools, like remote sensing, image analysis and -omics are expected to contribute to model development and validation, improving their performances in decision support systems, assisted by ICT solutions for stakeholders in a chain and multi-actor view.
.

FROM "ONE-FUNGUS ONE-CROP" APPROACH TO *ASPERGILLUS FLAVUS* AND *FUSARIUM VERTICILLIOIDES* JOINT PREDICTIVE MODEL

CAMARDO LEGGIERI M.*, GIORNI P., BATTILANI P.
Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, via E. Parmense 84, 29122 Piacenza, Italy.
marco.camardoleggieri@unicatt.it

The co-occurrence of mycotoxins in food/feed, the variability of toxin contamination between years and in the same year in close geographic areas, has become increasingly important. A critical role in this trend is played by climate change with the occurrence of extreme events at global and local level. These events seem strictly connected to the decreased reliability of model predictions, being the available models developed following the "one-fungus one-crop" approach. Consequently, it is essential to gain knowledge on the impact of fungi co-occurrence on growth and mycotoxin production in different ecological conditions. For this purpose, trials were carried-out both *in vitro* and in field with *Fusarium verticillioides* (*Fv*) and *Aspergillus flavus* (*Af*). *In vitro*, growth of *Af* and *Fv* decreased to 10% and 44% respectively, in case of co-inoculum, compared to single inoculum; toxin production dynamics showed a comparable trend with single or co-inoculum. In field, *Af* resulted dominant on *Fv* when co-inoculated and *Af* produced more AFs when *Fv* co-occurred; on the contrary, FB was greater when *Fv* occurred alone. These results contributed to develop Myco-maize, a joint model starting from AFLA-maize and FER-maize models. The validation showed a slight improvement in predictions, but it suggests further model performances improvements are possible. Plant-fungi interaction and cropping system were confirmed to play a role. Therefore, additional work is requested to include these aspects in the model.

THE POWER OF PREDICTION, EARLY WARNING AND ICT SOLUTIONS TO MITIGATE MYCOTOXIN RISKS

VAN DER LEE T. A. J.[1*], MOLENDIJK L. P.G .[1], AUDENAERT K.[2], S. LANDSCHOOT K.[2], VERWAEREN J.[2], LEGGIERI M. C.[3], BATTILANI P.[3], PERRONE G.[4], LOGRIECO A. F.[4], BEEN T. H.[1]

[1] *Wageningen University & Research, Wageningen, The Netherlands;* [2] *Ghent University, Ghent, Belgium;* [3] *Università Cattolica del Sacro Cuore, Piacenza, Italy;* [4] *National Research Council, Institute of Sciences of Food Production, Bari, Italy*

theo.vanderlee@wur.nl

Prediction and warnings are important for growers to timely respond to treats and optimize the growth of crops. The MycoKey app was developed to aid mycotoxin risk mitigation by growers, grain collectors, governmental planners and policy makers. The MycoKey app predicts the amounts of the most important mycotoxins in winter wheat (DON) and grain maize (Aflatoxin B1 and Fumonisin) based on local weather data and predictive models. It provides direct links to the scientific articles describing the models in detail. Both access to the platform and the MycoKey app is free of charge and user data are private. Enlist at https://akkerweb.eu/en-gb/ and download the free MycoKey app. Growers can calculate the predicted amount of mycotoxins in their crops with local weather information and relevant agronomic measures such as ploughing or the level of plant resistance based on a list of cultivars. Grain collectors, governmental planners and policy makers have access to public databases including satellite data, and can calculate mycotoxin risks based on weather data and land use (if publicly available). Recalculation allows integration of management strategies in the risk model and calculations of "what if" scenarios. The risk analysis was performed for the mycotoxin DON in winter wheat and in 2019 and 2020 DON mycotoxin risks for winter wheat were low in the Belgium and the Netherlands. In contrast in 2021 conditions were more conducive for Fusarium and the risks for DON were much higher particular in the northern part of the Netherlands. The Mycokey app was used to create awareness and helped to initiate responses in the chain to mitigate mycotoxin risks in the Netherlands. The early warning was made possible by filling the gap between forecast and harvest time using historic weather available for the last 40 years. These historic data can also be applied to assess the impact of weather in climate change scenarios. The need for an integrated approach to mitigate mycotoxin risks in crop management systems will be discussed.

This work was partly supported by the MycoKey-Project H2020-(E.U.3.2-678781)

to Index

Mycotoxins in Animal Production 2

Mycotoxin Control in Animal Feed

FEED SUPPLEMENTATION WITH PROBIOTICS AS A STRATEGY AGAINST THE LOW-DOSE EFFECTS OF DEOXYIVALENOL IN PIG

ALASSANE-KPEMBI I.[1]* , OSWALD I.P.[2]
[1]*Département de Biomédecine, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada;* [2]*Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toxalim, 31027, Toulouse, France.*
imourana.alassane-kpembi@umontreal.ca

Low-dose contamination by deoxynivalenol (DON) is a real feed safety issue. Pigs are particularly sensitive to this mycotoxin that disrupts normal cell functions. Some probiotics have demonstrated ability to tackle various factors threatening pig intestine health. We analysed on a tiered-approach the health effects of probiotics supplementation to piglets facing a subclinical DON challenge. Whole-transcriptome analysis on jejunal explants explored the early intestinal response to DON after administration of S. cerevisiae boulardii strain CNCM I-1079 (Scb), and in vivo trial investigated the intestinal and systemic effects of the mycotoxin and the yeast. Exposure to 10µM DON triggered 32 signaling pathways in the intestine while administration of yeast only induced no differentially expressed gene. Co-administration of Scb and mycotoxin reversed some of the prototypical inflammation pathways triggered by DON, including NF-κB and p38 MAPK, and also restored the antioxidant action of vitamin C and the lipid metabolism pathways. No clinical signs, nor significant modifications of blood biochemistry parameters were detected in piglets exposed to 3 mg/Kg DON. However, histological changes appeared in jejunum, liver and kidney samples. ^{1}H-NMR metabolomic profiling of plasma and liver samples unvealed alteration of amino acids and 2-oxocarboxylic acids metabolism. Yeast supplementation clearly mitigated the DON-induced histological lesions and restored the plasma metabolic profile. By contrast, the effect of Scb supplementation remained marginal on the liver metabolome, indicating that the toxicity of the mycotoxin was attenuated, but not abolished. These results support the detrimental effects of low-level contamination by DON, and indicate that supplementation with Scb increases piglet resilience to a subclinical challenge with the mycotoxin.

This study was financially supported by ANR grant ExpoMycoPig (Agence Nationale de la Recherche ANR-17-Carno12 France) and Project CLE2014 funded by La Région Occitanie (France) and Lallemand SAS.

A HIGH MYCOTOXIN CONTAMINATION IS EXPECTED IN EUROPEAN WHEAT EXPORTING COUNTRIES, INCREASING RISK FOR PIGLETS

PREVERAUD D.P.*, DVORSKA J., LECOLINET M.
Adisseo France SAS, Immeuble Antony Parc 2, 10 Place du Général de Gaulle, 92160 Antony, France
damien.preveraud@adisseo.com

Adisseo, in collaboration with Syngenta, have developed a practical new tool called MycoMan Predict, which allows to estimate the risk of crop contamination before the harvest. According to MycoMan Predict 2021, the deoxynivalenol (DON) contamination of wheat in certain major exporting countries, including Ukraine, Russia (the Black Sea area) and France is high. DON can cause negative effects on the health and performance of swine, the most sensitive among the animal species. Therefore, we have evaluated the effect of a natural contamination of DON in nursery pigs. A total of 18 barrows and 18 gilts, weaned at 27 days of age, were allotted for 34 d to 3 dietary treatments: M- diet with corn DDGS containing minimal amounts of detectable DON; M+ diet with corn DDGS contaminated with 2 mg/kg DON; UP diet M+ supplemented with 0.2% Unike® Plus. The M+ diet compromised the ADG by -11.7%, and the ADFI by -5.9%, and thus lowered G:F ratio by -5.6%. UP restored ADG by +4.2% and G:F by +3.0%, compared to M+. The M+ diet affected the intestinal health of the animals by modulating the redox parameters (GSH/GSSG and MDA), inflammatory (IL8) and immune response (IgA). The pigs fed with UP had a higher (P=0.036) globulin concentration in the serum. The supplementation of Unike® Plus to the pig diet also led to an increase in GSH/GSSG (P=0.045) and a decrease of MDA (P=0.037) concentrations in the jejunum mucosa. IL-8 and IgA were also decreased in the UP group compared to the M+ group. Solutions that have a complementary approach to adsorption, bio-inactivation, support of immunity, organs and antioxidative protection seem to be more promising to offset the negative effects caused by mycotoxins.

EFFICACY ASSESSMENT OF YEAST FRACTIONS IN ADSORBING MYCOTOXINS AND IN REDUCING THEIR ORAL BIOAVAILABILITY IN ANIMALS.

MARQUIS V.[1], D'ASCANIO V.[2], CATTEUW A.[3], GRECO D.[2], CROUBELS S.[3], LOGRIECO A.F.[2] AVANTAGGIATO G[2]

[1]Phileo by Lesaffre, Rue Gabriel Péri 137, 59700 Marcq-en-Baroeul, France; [2]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Giovanni Amendola, 122/O, 70126 Bari, Italy; [3]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

In addition to their efficiency as postbiotics, delivering probiotic effects on microbiota and immunity, yeast cell walls (YCW's) are widely used by the animal feed industry as mycotoxin adsorbents. Several studies have shown that most YCW's, when tested *in vitro*, display evident mycotoxin reducing effects, but with differing levels of consistency. Our focus, therefore, was directed to the selection of a yeast cell wall with high adsorbing capacities for mycotoxins.
After selecting an effective YCW based on *in vitro* adsorption results, toxicokinetic studies were performed in rats and piglets to investigate the efficacy of YCW in reducing the systemic exposure of mycotoxins.
The effect of the mycotoxin detoxifier on the oral absorption of the mycotoxin was evaluated by statistical comparison of toxicokinetic parameters between control and treated groups, with special emphasis on area under the curve from time zero to infinite ($AUC_{0\rightarrow\infty}$), maximal plasma concentration (C_{max}), time at maximal plasma concentration (T_{max}) and relative oral bioavailability (F).
The YCW product tested in *in vivo* experiments was able to reduce significantly the intestinal absorption of mycotoxins tested.

This research was financially supported by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey)

DEGRADATION OF MYCOTOXINS USING BACILLUS SP.

RAJ J.*, JAKOVČEVIĆ Z., FARKAŠ H., BOŠNJAK-NEUMÜLLER J., VASILJEVIĆ M.
PATENT CO, DOO., Vlade Ćetkovića 1A, 24 211, Mišićevo, Serbia
jog.raj@patent-co.com

Mycotoxins are the biggest challenge for animal feed producers and can be remediated by various methods but enzymes and microbes for deactivating mycotoxins are becoming popular these days. In this study different microbes were screened for degradation of mycotoxins. On day 1, a culture of microbe was inoculated in nutrient broth or mineral salt medium and incubated at 370C for 16-20 hours. The next day DON, ZON, AFB1, FB1, OTA and T-2 mycotoxins were added to the flask to make a final concentration at 0.2 ppm and was called Test flask. Control flask containing culture medium and 0.2 ppm of toxins and Test flask containing couture medium, microbes and mycotoxins were incubated at 370C and samples were taken at 0, 2, 6, 12 and 24 hours for analysis of degradation activity using LC-MS/MS. The results showed that Bacillus sp. in 2 hours resulted in 26 % transformation of AFB1 to AFQ1, 25% transformation of OTA to OTA Alpha and OTA B (very low detected levels), and 95% transformation of ZON to alpha-Zearalenol but only 39 ppb was detected and this level of alpha-Zearalenol does not have toxic effects.

REDUCTION OF ADVERSE EFFECTS OF LOW MULTIPLE TOXINS CONTAMINATED FEED IN PIGLETS BY ANTI-BIOTOXINS SUPPLEMENTATION

TECHER C.* AND GUILLOU D.
1Mixscience, 2/4 Avenue de Ker Lann, CS 17228 35172 BRUZ CEDEX France.
clarisse.techer@mixscience.eu.

The effect of low quantities of Zearalenone (ZEN) and their potential interaction with other mycotoxins is little described in piglet. This study aimed to evaluate the impact of low mycotoxins contaminated feed in piglets and the effect of anti-biotoxin supplement (MPY) on animal growth. Trial was performed with 160 piglets, comparing 4 diets in the post-weaning period, designed as 2X2 factorial: control (T) or low contaminated maize (FC) with or without supplement (MPY). In addition to growth performance parameters, blood biomarkers were performed for 40 piglets and tentative measurements of vulva size of female piglets was undertaken in 3 pens (diets T, FC, FC+MPY). Feed mycotoxins analysis showed level of contamination for DON and ZEN of 0.245 and 0.020 mg/kg for the control diet. Higher values, i.e 0.480 and 0.050 mg/kg, respectively for DON and ZEN, have been measured in FC maize diet. All diet expressed naturally low level of fumonisin B1, T2, HT-2 and ergot alkaloids. Results showed that piglet health and performance were not affected by treatments, despite a weak trend ($P<0.15$) for higher final live weight, improving also feed efficiency for MPY diet ($P<0.05$). Among blood biomarkers, alanine amino transferase was increased by FC ($P<0.001$), indicating stimulated liver detoxication pathways. Length of piglet's vulva was increased by 24% comparing FC to T, MPY addition limited this growth to +11%. This study confirmed low but measurable adverse effects of cocktail of low dose of mycotoxins fed to post-weaning piglets and the possibility to alleviate their effect with MPY supplementation.

FEED ADDITIVES FOR THE REDUCTION OF THE CONTAMINATION OF FEED BY MYCOTOXINS: AUTHORISATION AND CONTROL METHODS WITHIN THE EUROPEAN UNION

VON HOLST C.
European Commission, Joint Research Centre, Retieseweg 111, B-2440 Geel, Belgium
Christoph.von-Holst@ec.europa.eu

Feed additives play an important role in animal nutrition, addressing different aspects such as feed safety, reduction of environmental emissions and sustainability of food production. Furthermore, these products are classified in corresponding categories and functional groups. Prior to placing these products on the European Union (EU) market, industry needs to apply for authorisation as specified by Regulation (EC) No 1831/2003. The procedure includes an assessment conducted by the European Food Safety Authority, the evaluation of analytical methods suitable for official control done by European Union Reference Laboratory (EURL) and the decision on the authorisation by the European Commission. In 2009, the European Commission introduced a new functional group of feed additives, comprised of 'substances for reduction of the contamination of feed by mycotoxins'. The purpose of the use of these additives is to gain a favourable effect of feed containing specific mycotoxins at concentrations even below the legal limit. Currently, there are various feed additives authorised under this group, targeting at the reduction of trichothecenes, aflatoxin B_1 or fumonisin. The Regulations authorising these products also contain references to specific methods of analysis - as evaluated by the EURL - that need to be applied by Member States' official laboratories to monitor correct levels of the products in feed or to check compliance with target characteristics of these products. The purpose of the presentation is to give an overview of this specific group, focusing on corresponding methods of analysis.

CHARACTERIZATION AND EFFECTIVENESS OF DURIAN PEEL AS A MULTI-MYCOTOXIN ADSORBENT

ADUNPHATCHARAPHON S.[1], PETCHKONGKAEW A.,[1] GRECO D.[2], D'ASCANIO V.[2], VISESSANGUAN W.[3], AVANTAGGIATO G.[2*]

[1]School of Food Science and Technology, Faculty of Science and Technology, Thammasat University, PathumThani, Thailand; [2]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Amendola 122/O, Bari, Italy; [3]Food and Feed Innovation Center, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), PathumThani, Thailand.

giuseppina.avantaggiato@ispa.cnr.it

Durian peel (DP) is an agricultural waste that is widely used in dyes, and for organic and inorganic pollutant adsorption. In this study, durian peel was acid treated to enhance its mycotoxin adsorption efficacy. This acid-treated durian peel (ATDP) was assessed for simultaneous adsorption of aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), and fumonisin B_1 (FB_1). Adsorption experiments were performed on ATDP at 0.5% (w/v) dosage, using multi-mycotoxin solutions containing 1 µg mL-1 of each toxin, in media of pH 3 and 7. The structure of the ATDP was characterized by scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, and surface-area analyzer. ATDP exhibited the highest mycotoxin adsorption towards AFB1 (98.4%), ZEA (98.4%), and OTA (97.3%), followed by FB1 (86.1%), and DON (2.0%). The pH significantly affected OTA and FB1 adsorption, whereas AFB1 and ZEA adsorption was not affected. Structural characterization showed more cavities in the ATDP surface compared to the untreated material (DP). C and O were the major elements on its surface. Acid treatment of DP changed the functional groups and charge of the adsorbent material. ATDP showed higher Brunauer-Emmett-Teller (BET) pore volumes, pore diameters, and BET surface area. These structural changes following acid treatment may explain the higher efficacy of ATDP in adsorbing mycotoxins. Hence, ATDP can be considered as a promising waste material for mycotoxin biosorption.

This research was funded by Thammasat University Research Fund, Contract No. TUIN 3/2562. The work was also supported by the MycoKey Project (European Union Horizon 2020, Research and Innovation Programme) under Grant Agreement No.678781.

ADSORPTION OF DEOXYNIVALENOL IN ANIMAL FEED BY A HYDROLYSATE OF A NOVEL STRAIN OF *SACCHAROMYCES CEREVISIAE*

BRUINENBERG P.[1]* AND CASTEX M.[2]

[1]Trouw Nutrition R&D, Stationstraat 77, 3811 MH Amersfoort, The Netherlands; [2]Lallemand Animal Nutrition R&D, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France.

paul.bruinenberg@trouwnutrition.com

Mycotoxicoses are diseases caused by exposure of animals to feeds contaminated with mycotoxins. One strategy to cope with mycotoxins is the use of adsorbing agents to reduce the exposure to mycotoxins by decreasing their bioavailability, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs. However, for tricothecenes (deoxynivalenol, T2-toxins), no efficient binders are available. In this study we describe an enzymatic and chemical hydrolysate of a selected strain of Saccharomyces cerevisiae that is able to adsorb mycotoxins, including deoxynivalenol (DON), in feed. These yeast products were tested for their in vitro binding capacity of various mycotoxins at pH 3.0, 5.0 and 8.5, mimicking the gastric passage of the binder. The results showed that these yeast products were able to bind DON and to a lower extend zearalenon (ZEA). Next we have determined the effect of the two yeast products on oral absorption of DON, ZEA and ochratoxin A (OTA) in pigs. For this purpose we used the in vivo kinetic model as developed by Devreese et al. (2014, Toxins, 6, 2998-3004). Pigs (± 20 kg) received an oral bolus of 3 mycotoxins (DON and OTA, 0.05 mg/kg BW; ZEA, 0.5 mg/kg BW) alone or in combination with the 2 selected yeast products (100 mg/kg BW). Toxicokinetic modeling of the plasma concentration-time profiles of DON, OTA and ZEA-GlcA showed that both yeast binders reduced the relative oral bioavailability and thus the systemic exposure of DON, OTA and ZEA-GlcA.

DEVELOPMENT OF A NEW BIO-ORGANOCLAY FOR MYCOTOXIN DECONTAMINATION: IN VITRO AND IN VIVO EVIDENCE

D'ASCANIO V.[1], GRECO D.[1], CATTEUW A.[2], CROUBELS S.[2], DEVREESE M.[2], SCALA R.[3], MAQOUD F.[3], TRICARICO D.[3], MENICAGLI E.[4], LOGRIECO A.F.[1], AVANTAGGIATO G.[1*].

[1]Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (CNR-ISPA), Bari, Italy; [2]Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; [3]Dipartimento di Farmacia-Scienze del Farmaco, Università degli studi di Bari Aldo Moro, Bari, Italy; [4]Laviosa Chimica Mineraria SpA, Livorno, Italy;

giuseppina.avantaggiato@ispa.cnr.it

Bentonites adsorb preferably aflatoxins, with little adsorption efficacy towards other mycotoxins. To overcome this limitation a bio-organoclay, acting as a multi-mycotoxin adsorbent, was developed by functionalization of a Na-smectite with an organic, non-toxic modifier. The process was optimized at lab and industrial level. At low dosages (0.25-0.5% w/v), the bio-organoclay sequestered more than 95% of AFB_1, FB_1, OTA, and ZEA, in a large range of pH values (3-9). Mycotoxin adsorption occurred simultaneously with high capacity and affinity as determined by equilibrium isotherms.

The efficacy of the bio-organoclay in reducing the systemic exposure to AFB_1, FB_1, OTA, and ZEA was further studied in rats and piglets, using the biomarker for exposure approach. Mycotoxins were administered by an intragastric oral bolus, singularly in rats and as a mixture in piglets. Control animals received the mycotoxins without the detoxifier, while treated animals received the mycotoxins with the bio-organoclay at 0.5% w/w of feed consumption. Samples of urine in rats and of blood in piglets were collected at different time points (4-72h), and then analysed for mycotoxin content by UPLC-FLD/PDA and UPLC-MS/MS methods, respectively. Toxicokinetic parameters, including area under the curve and maximal mycotoxin concentration, were calculated and used to compare control and treated groups. The bio-organoclay significantly reduced urinary excretion of AFM_1, ZEA, FB_1 and OTA in rats. In piglets, it was significantly effective in reducing systemic exposure to AFB_1 and OTA, while the reduction of ZEA and FB_1 exposure was not significant.

In conclusion, the high efficacy of the bio-organoclay in sequestering AFB_1, ZEA, OTA and FB_1 measured by isothermal adsorption studies was fully and partially confirmed by rat and piglet studies, respectively.

This research was financially supported by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey); and by the research agreement between CNR-ISPA and Laviosa Chimica Mineraria SpA (Prot. No. 3123 on 27/06/2016)

GEOLOGICAL ORIGIN OF BENTONITE: ITS ROLE IN THE SELECTION OF POTENTIAL BINDERS FOR AFLATOXIN ADSORPTION

D'ASCANIO V.[1], GRECO D.[1], MENICAGLI E.[2], LOGRIECO A.F.[1], CATUCCI L.[3], AVANTAGGIATO G.[1].

[1]Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (CNR-ISPA), Bari, Italy; [2]Laviosa Chimica Mineraria SpA, Livorno, Italy. 3Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari, Italy.

giuseppina.avantaggiato@ispa.cnr.it

Since 2013, bentonite in the form of dioctahedral smectite is an additive authorised in the EU as a substance for the reduction of the contamination of feed by aflatoxins. Several studies indicate a big difference in the effectiveness of bentonites in sequestering aflatoxins. A clear correlation between mineralogical and physico-chemical properties of bentonites and aflatoxin adsorption has not been well established. In this study, the most critical mineralogical, chemical, and physical properties that affect aflatoxin adsorption by bentonite were evaluated. Bentonite samples (29), mined from different locations around the world, were analyzed against the published selection criteria for aflatoxin adsorbents: mycotoxin adsorption parameters (maximum adsorption capacity and affinity) determined by the method of adsorption isotherms; pH; cation exchange capacity; particle size distribution; mineralogical/structural compositions; swell index and viscosity. A correlation between geological origin and AFB_1 adsorption capacity was found (p<0.001). Sedimentary bentonites were significantly more effective than hydrothermal ones in adsorbing aflatoxin at different pH values. The extent of AFB_1 adsorption by all samples was negatively and linearly correlated to the extent of desorption, and sedimentary bentonites were significantly more effective than hydrothermal ones in keeping bound the adsorbed fraction of the toxin (p<0.001). In addition, AFB_1 adsorption by bentonites correlated positively with sodium content and swell index, but negatively with d001-value, magnesium and calcium contents. In conclusion, it seems that the geological origin of bentonite is a useful guide for the selection of a good binder for AFB_1 reduction. Sedimentary bentonites containing sodium/swelling -smectite should be preferred to hydrothermal samples as potential aflatoxin binders. Taking into account the geographical origin of our samples, this approach should be applicable to bentonites worldwide.

This research was financially supported by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey); and by the research agreement between CNR-ISPA and Laviosa Chimica Mineraria SpA (Prot. No. 3123 on 27/06/2016)

REDUCTION OF MYCOTOXINS BY BRUSHING TECHNIQUE

DJURAGIC O.[1], COLOVIC R.[1], BAGI F.[2], BURSIC V.[2], STOJSIN V.[2]
[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; [2]Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovica 8, 21000 Novi Sad, Serbia
olivera.djuragic@fins.uns.ac.rs

Mycotoxins are a worldwide problem, causing many damages to animal and human health through the food chain. When mycotoxins enter the food chain, it is very difficult to eliminate them since there is a limited number of methods for action. Those methods could be divided into three main groups: chemical, biological, and physical. Physical methods could be very effective in grain processing, depending on the type of mycotoxin and its distribution through the kernel. Aflatoxin is usually present on the surface of the kernel as well as in the germ since Deoxinyvalenol (DON) is distributed through the whole kernel. Physical methods for reduction of the content used in grain processing are usually cleaning, polishing, mechanical sorting and separation, milling washing, density segregation, flotation, and thermal treatment. Laboratory maize brusher was developed at the Institute of Food Technology, Novi Sad for cleaning and polishing of maize kernel surface in research purposes. Laboratory brushing was effective in reducing fumonisin content from 3-73% at levels of fumonisin content from 15-78 μg/g ($p < 0.05$). The application of the brushing process had a significant influence ($p < 0.05$) on reducing the content of aflatoxin B1. The use of brushes for corn kernel surface cleaning induced the reduction of the specified mycotoxin content from 57.0 to 98.7%. while aflatoxin B2 was reduced from 36% to 99.6%, depending on the sample. Brushing process is proved to be effective in mycotoxin removal, with no negative effects on physical characteristics of maize kernel.

This study has been supported by the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina through the project "Application of novel and conventional processes for removal of most common contaminants, mycotoxins and salmonella, in order to produce safe animal feed in the territory of AP Vojvodina", Project No. 142-451-2478/2018-01/02

ZEARALENONE BIODEGRADATION BY MICROBIAL STRAINS ISOLATED FROM CORN AND WHEAT EARS

EVANGELISTA A.G., PINTO A. C. S. M., ORSO P. B.,. CORRÊA J. A. F, F. B. LUCIANO*.
Graduate Program in Animal Science, Pontifícia Universidade Católica do Paraná, 1155 Rua Imaculada Conceição, 80215-901, Curitiba, Brazil
fernando.luciano@pucpr.br

Zearalenone (ZEA) is a mycotoxin with estrogenic activity produced by *Fusarium* strains. Interaction between fungi and bacteria in plants may lead to evolutionary adaptations, and some microorganisms develop the ability to degrade ZEA. This study sought the potential biodegradation of ZEA by combinations of bacteria isolated from corn and wheat ears. Plants were collected in Southern Paraná, Brazil. After isolation, preliminary esterase activity assay, predictive for ZEA biodegradation, revealed several bacterial strains with potential for biocontrol of this mycotoxin. Strains with highest potential, named 62 (64%), 178 (78%) and 197 (99%), were next identified as *Bacillus*. Overnight cultures of each bacteria were prepared with or without agitation (150 rpm) at 30 ºC. Rightafter, 1 mL broth contaminated 1 µL/L of ZEA were inoculated with either of the following combinations: C1: Pool of all strains, with agitation; C2: Pool of all strains, without agitation; C3: 62 and 197, with agitation; and C4: 178 and 197, with agitation. Combinations were incubated for 24 h, 30 ºC, 120 rpm. Then, ZEA was extracted with ethyl acetate and analyzed by HPLC-FLD. C1, C2, C3 and C4 promoted degradations of 26.7, 91.0, 56.3 and 55.0%, respectively. The best activity was observed in C2, which the bacteria were initially cultivated without agitation. Combination of bacteria did not increase the level of ZEA degradation, but substantial levels of the mycotoxin are still reduced. Combined strains could be a better approach for biodegradation, since higher rates of plant adherence may be reached with a formulation containing genetic variability.

The authors thank the Brazilian National Council for Scientific and Technological Development (CNPq, Brazil) for financial support (Process 400896/2014-1) and scholarship (Process 142196/2019-3).

SMALL-SCALE PRODUCTION OF DEOXYNIVALENOL (DON) FOR RAPID DETOXIFICATION ASSAYS

EVANGELISTA A.G., M PINTO A. C. S., LUCIANO F. B.
[1] School of Life Sciences, Pontifícia Universidade Católica do Paraná, 1155 Rua Imaculada Conceição, Prado Velho 80215-901, Curitiba, Brazil.

Deoxynivalenol is a toxic metabolite produced by Fusarium sp. in the field that provokes emesis and decrease of feed intake affecting negatively animal performance and therefore animal production industry. This work aimed to establish a small-scale system to produce deoxynivalenol for screening microorganisms able to degrade DON. Five strains of Fusarium graminearum (CECT-2150, ITEM 126, 6352, 6415, and 5065) were cultivated in PDA for 5 days at 25 °C. After this period, 5 mL of peptone water (0,1%) were added on the grown colonies, which were scraped to form a spore suspension and adjusted to 10⁶ conidia/mL. 200g of corn were hydrated with 200 mL of water and sterilized (121 °C). After cooling, fungal suspensions were inoculated in the jar and cultivated for 21 d under 250 RPM and 30 °C. After this period, the jar was sterilized. Toxin extraction was performed with 20 mL of methanol P.A. and 5g of corn, homogenized in turrax per 5 min. The extract was filtered, evaporated (50 °C), resuspended in 1 mL of methanol HPLC grade, and analyzed by HPLC-DAD, using as stationary phase C18 column, reversed-phase and isocratic mobile phase composed by ultrapure water and acetonitrile HPLC grade (90:10) at a flow of 0.8 mL/min at 40 °C, with absorbance reading at 220 nm. Two strains produced the toxin, strains 6352 and CECT-2150 produced 1600 µg/g and 500 µg/g of DON, respectively, and these extracts were successfully used to contaminate samples in further tests decreasing the experiment costs related to purified toxin.

This work was supported by the Brazilian National Council for Scientific and Technological Development, process CNPq 308598/2020-2 and 437728/2018-8; the Coordination for the Improvement of Higher Education Personnel, processes CAPES 001; and the Pontifical Catholic University of Parana.

AGRICULTURE WASTE MATERIALS AS POTENTIAL MYCOTOXIN ADSORBENTS

GRECO D., D'ASCANIO V., AVANTAGGIATO G.
Istituto di Scienze delle Produzioni Alimentari, Via Amendola 122/O, Bari, Italy.
giuseppina.avantaggiato@ispa.cnr.it

In the field of animal protection, the use of adsorbent materials to decontaminate mycotoxin contaminated diets will become an effective strategy to counteract mycotoxin problems in animal production if these materials have the ability to bind combinations of mycotoxins. So far, most substances used as mycotoxin binders (in particular clays) fail in sequestering structurally different mycotoxins. This study examined the ability of 51 agricultural-by products to adsorb mycotoxins from liquid mediums simulating the pH values (3 and 7) that can be found in the GI tract of humans and monogastric animals. Mechanism of mycotoxin adsorption was studied by isotherm adsorption experiments. Grape pomaces, artichoke wastes, and almond hulls were selected as promising biosorbents, being quite effective towards AFB_1, ZEA, and OTA. Their adsorption was not affected by medium pH, and the adsorbed fraction was not released when pH rose from acid to neutral values. FB_1 was adsorbed to a lesser extent, while DON was not adsorbed. For selected agricultural by-products, maximum adsorption capacities calculated by the Freundlich, the Langmuir, and the Sips isotherms ranged from 1.2 to 2.9 μg/mg for AFB_1, 1.3 to 2.7 μg/mg for ZEA, 0.03 to 2.9 μg/mg for OTA, and 0.01 to 1.1 μg/mg for FB_1.
In conclusion, this study shows that selected agricultural by-products can find technological applications as feed/food additives for mycotoxin reduction. They represent a low cost, and potentially valuable source of phenolic antioxidants and undegradable fibre, which can promote health also through their ability to "trap" mycotoxins in the digestive tract.

METABOLISM OF ZEARALENONE IN THE RUMEN OF DAIRY COWS WITH AND WITHOUT APPLICATION OF A ZEARALENONE-DEGRADING ENZYME

GRUBER-DORNINGER C.*, FAAS J., DOUPOVEC B., ALESCHKO M, HOEBARTNER-GUSSL A., SCHOENDORFER K., KILLINGER M., ZEBELI Q.2 SCHATZMAYR D.

BIOMIN RESEARCH Center, 3430 Tulln, Austria. 2Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

christiane.gruber-dorninger@dsm.com

The estrogenic mycotoxin zearalenone (ZEN) is a frequent contaminant of animal feed. Ruminants are generally considered less sensitive to ZEN than pigs. However, ZEN was found to be metabolized to the highly estrogenic compound α-zearalenol (α-ZEL) in rumen liquid *in vitro*. In this study we aimed to investigate the metabolism of ZEN in the rumen of fistulated dairy cows (Gruber-Dorninger et al.; Toxins 2021, 13, 84). Cows received an oral dose of ZEN (5 mg ZEN in 500 g feed). Subsequently, concentrations of ZEN and ZEN metabolites were analyzed in free liquid obtained from three rumen compartments (ventral sac, reticulum, dorsal mat) 15 min to 34 hours after ZEN administration. α-ZEL was the predominant ZEN metabolite detected in every compartment. In addition, lower concentrations of β-zearalenol (β-ZEL) were detected. ZEN, α-ZEL and β-ZEL were eliminated from ventral sac and reticulum within 34 h, while in the dorsal mat low levels of ZEN and α-ZEL were still detected 34 h after ZEN administration. In a second step, we evaluated the efficacy of the enzyme zearalenone hydrolase (ZenA) added to ZEN-contaminated feed to degrade ZEN in the rumen. Upon administration of ZenA, ZEN, α-ZEL and β-ZEL concentrations in rumen liquid were significantly reduced and the non-estrogenic metabolites hydrolyzed zearalenone (HZEN) and decarboxylated HZEN were detected. In conclusion, the highly estrogenic compound α-ZEL was the predominant metabolite of ZEN formed in the rumen of dairy cows. ZenA applied as a feed additive may be a promising strategy to counteract estrogenic effects of ZEN.

EFFICACY ASSESSMENT AND PROTECTIVE EFFECT OF YEAST FRACTIONS IN REDUCING THE NEGATIVE IMPACT OF MYCOTOXINS IN ANIMALS

MARQUIS V.[1], D'ASCANIO V.[2], CATTEUW A.[3], GRECO D.[2], CROUBELS S.[3], LOGRIECO A.[2] AVANTAGGIATO G.[2]

[1]Phileo by Lesaffre, Rue Gabriel Péri 137, 59700 Marcq-en-Baroeul, France; [2]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Giovanni Amendola, 122/O, 70126 Bari, Italy; [3]Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

v.marquis@phileo.lesaffre.com

This presentation aims to shed light on yeast-based solution for mycotoxin mitigation. Considerable research has been conducted to evaluate the potential animal growth performance and health benefits of adding yeast, yeast-derivatives, and yeast-containing ingredients into animal feeds (Shurson, 2018). In particular, supplementing animals with feed containing yeast cell wall (YCW) derivatives has shown positive results on elimination of mycotoxins and inhibition of their toxic effects.

The use of YCW derivatives as mycotoxin binders can be considered a promising approach to protect animals against the harmful effects of mycotoxins contaminated feed. Several *in vitro* studies confirmed the potential of YCW in binding mycotoxins. As *in vitro* studies do not always predict *in vivo* results, *in vivo* experiments are required. Here, in order to investigate the efficacy of a YCW in reducing the systemic exposure of zearalenone (ZEN), a toxicokinetic study was performed in piglets focusing on plasma concentration-time profiles of ZEN-glucuronide. The YCW product tested in *in vivo* experiments based on the European Food Safety Authority, was able to reduce significantly the intestinal absorption of ZEN in piglets.

In addition, yeast and YCW are immunomodulatory compounds that interact directly and indirectly with pathogens and components of the immune system. Hence yeast-based products, thanks to their immunomodulatory properties as well as their ability to maintain a favorable and healthy intestinal environment, contribute to reduce the toxic effects of mycotoxins.

This research was financially supported by the European Union's Horizon2020 Research and innovation programme under Grant Agreement No.678781 (MycoKey)

MYCOTOXIN OCCURRENCE IN BREAST MILK AND RISK CHARACTERIZATION OF LACTATING MOTHERS USING URINARY BIOMARKERS IN SÃO PAULO, BRAZIL

COPPA C.F.S.C.[1], CIRELLI A.C.[1], GONÇALVES B.L.[1], BARNABÉ E.M.B.[2], PETTA T.[3], FRANCO L.T.[1], KHANEGHAH A.M.[4], ROSIM R.E.[1], LEE S.H.I.[1], CORASSIN C.H.[1], OLIVEIRA C.A.F.[1*]

[1]Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte, 225, CEP 13635-900, Pirassununga, SP, Brazil; [2] Unidade Materno-Infantil, Centro de Especialidades Médicas, Av. Antônio Joaquim Mendes, 1017, CEP 13634-502, Pirassununga, SP, Brazil; [3]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Do Café, s/n, CEP 14040-903, Ribeirão Preto, SP, Brazil; [4]Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz - Barão Geraldo, CEP 13083-970, Campinas, São Paulo, Brazil.
carlosaf@usp.br

This study aimed to evaluate the occurrence of mycotoxins in breast milk and in urine from 74 lactating women in Pirassununga, São Paulo, Brazil, and characterize the associated risk based on urinary biomarkers. Samples were collected from April/2018 to August/2019, and the mycotoxins were determined by ultra-performance liquid chromatography-tandem mass spectrometry. Aflatoxin M_1 (AFM_1) was not detected in any sample of breast milk, although 3 samples contained ochratoxin A (OTA) and 2 samples had fumonisin B_1 (FB_1) at concentrations of 0.018-2.6 ng/mL and 3.4-6.6 ng/mL, respectively. The median urinary levels of AFM_1 deoxynivalenol (DON), OTA and zearalenone (ZEN) were 0.16 (*n* = 42, 57%), 38.59 (*n* = 13, 18%), 2.38 (*n* = 6, 8%) and 0.64 ng/mg creatinine (*n* = 7, 10%), respectively. However, FB_1 was not detected in any urine sample. The mean probable daily intake (PDI) of aflatoxin B_1 (AFB_1), DON, OTA and ZEN, based on the urinary levels of their respective biomarkers and excretion rates described in the literature, were 1.58 ± 6.84, 1.09 ± 0,71, 5.07 ± 8.00 and 0.05 ± 0,06 µg/kg body weight/day, respectively. Hazard quotient (HQ) values > 1 were observed for OTA (316.8) and DON (1.1), indicating a non-tolerable risk. The Margin of Exposure (MoE) calculated for AFB_1 was 1.50, which represents a potential health concern (MoE < 10,000). Although a low incidence of mycotoxins was observed in breast milk, the urinary levels of mycotoxin biomarkers indicated a high exposure of lactating women to dietary mycotoxins in the studied area.

This research was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Grant # 2017/12956-2.

MYCOTOXIN CONTAMINATION OF ECUADORIAN FOODS WITH HIGH NUTRITIONAL VALUE

ORTIZ-ULLOA J.,CASTRO M., SAQUICELA J., ASTUDILLO G., DONOSO S.
Department of Biosciences. "Food, Nutrition and Health" Research Unit. Faculty of Chemical Sciences. Cuenca University. Av. 12 de Abril s/n, 010203-Cuenca, Ecuador.
johana.ortiz@ucuenca.edu.ec

In Ecuador, there is special interest in promoting the consumption and production of ancestral crops and foods with minimal processing to preserve their high nutritional value. Although those foods are produced in sufficient amounts, storage capacity and conditions are not always optimal and safe. This study aimed to evaluate mycotoxin contamination of some of these foods: ochratoxin A (OTA), fumonisin B1 (FB1) and aflatoxins (AFs) in brown rice and quinoa; OTA, FB1 and deoxynivalenol (DON) in whole wheat flour, and OTA, FB1 and AFs in lupine beans. Samples were collected in bulk from two Andean cities and one coastal city of Ecuador. AFs, OTA and DON were extracted with methanol/water 80:20 (v/v), acetonitrile/water 60:40 (v/v) and pure water, respectively. The extracts were purified and analysed by RP-HPLC with fluorescence detection for AFs, FB1 and OTA, and with UV detection for DON. Contamination profiles were different among the studied cities. Although, warm and humid climate, in the coastal city no contamination with mycotoxins in any food above the maximum permissible limit (MPL) was observed. In the Andean cities, whole wheat flour was contaminated with FB1 (47% >MPL) and DON (60% >MPL); lupine beans with OTA (13% >MPL) and quinoa with OTA (38% >MPL). Mycotoxin co-occurrence in different high-nutritional value foods in Ecuador was evidenced as well as different contamination profiles among regions. Further actions to monitor, prevent and mitigate mycotoxin contamination at pre- and post-harvest levels should be developed towards friendly adoption by the community.

This research was financially supported by the Project CEPRA-XI-2017-02: Fortalecimiento de la inocuidad alimentaria en el Ecuador: Estudio de los contaminantes microbianos, micotóxicos y de metales pesados en alimentos y establecimiento de un sistema nacional de capacitación en línea.

2019 SURVEY OF MYCOTOXINS IN WHEAT

BORUTOVA R.[1], PREVERAUD D. P.[2], AVERKIEVA O.[1]
[1]Adisseo, Hoogveld 93, 9200 Dendermonde, Belgium ; [2]Adisseo France SAS, Antony Parc 2, 10 Place du Général de Gaulle, 92160 Antony, France.
radka.borutova@adisseo.com

The survey included 117 wheat samples from Poland and provided insight into the incidence of aflatoxin B1 (AfB1), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin, HT-2 toxin, fumonisin B1 (FB1), fumonisin B2 (FB2) and ochratoxin A (OTA). Samples were collected in 2019 directly from farms or animal feed production sites according to the principles of good sampling (Richard, 2000) immediately after harvesting to avoid storage mycotoxins development. Mycotoxins were analyzed by LC MS/MS. For the purpose of data analysis, non-detection levels were based on the limits of quantification of the test method for each mycotoxin.

The results showed that 29% of the samples were contaminated with DON and only 3 % contained ZEN. The maximum concentration of DON was 2300 µg/kg (8X higher than in 2018) and 400 µg/kg for ZEN. Few samples (1%) were contaminated with OTA and the maximum concentration was also low (45.5 µg/kg). 56% of the samples were contaminated with HT-2 toxin and the maximum concentration recovered was 283 µg/kg. None of the samples contained AfB1 and FB2. 3% of the samples were contaminated with FB1 and the maximum concentration was 3750 µg/kg. The average number of positive samples in 2019 was approximately 2.5X those of 2017 and 5X higher than in 2018.

The mycotoxin survey concluded that the wheat harvest was of medium quality in terms of mycotoxin contamination. Therefore, wheat from Poland should not automatically be considered safe for inclusion in finished feed rations for all animal species and a degree of vigilance is prudent.

2019 SURVEY OF MYCOTOXINS IN MAIZE

BORUTOVA R.[1], PREVERAUD D. P.[2], AVERKIEVA O.[1]
[1]Adisseo, Hoogveld 93, 9200 Dendermonde, Belgium ; [2]Adisseo France SAS, Antony Parc 2, 10 Place du Général de Gaulle, 92160 Antony, France.
radka.borutova@adisseo.com

This survey on maize samples from Brazil provided insight into the incidence of aflatoxin B1 (AfB1; n=959), zearalenone (ZEN; n=281), deoxynivalenol (DON; n=305), T-2 toxin (n=210), HT-2 toxin (n=196), fumonisin B1 (FB1; n=325), fumonisin B2 (FB2; n=324) and ochratoxin A (OTA; n=181). Samples were collected in 2019 directly from farms or animal feed production sites according to the principles of good sampling (Richard, 2000) immediately after harvesting to avoid storage mycotoxins development. Mycotoxins were analyzed by LC MS/MS.

The results showed that 93.8% of the samples were contaminated with FB1 with maximum concentration of 10 224 µg/kg and average concentration of 1085.3 µg/kg. The results also showed that 75.3% of the samples were contaminated with FB2 with maximum concentration was 3086 µg/kg. Only 3.3 % of samples contained DON. None of the samples were contaminated with OTA and none of the samples contained T-2 toxin and HT-2 toxin. 12% of the samples were contaminated with AfB1 with highest concentration was 251 µg/kg. The maximum concentration of ZEN recovered was 1399 µg/kg. While the results for AfB1 show similar trend, the results of FB1 show that the average concentration of positive samples was significantly lower in 2019 than in 2018.

This survey concluded that the harvest in Brazil was of medium to low quality in terms of mycotoxin contamination and so, the 2019 maize crop should not automatically be considered safe for inclusion in finished feed rations for all animal species and a degree of vigilance is prudent.

AN INNOVATIVE AND ACCURATE PREDICTION MODEL TO MITIGATE THE RISK OF MYCOTOXINS.

PREVERAUD D. P.[1], FROMENT A.,[2] DVORSKA J[1].
[1]*Adisseo France SAS, Antony Parc 2, 10 Place du Général de Gaulle, 92160 Antony, France;*
[2]*Syngenta, 1 Avenue des Prés, 78286 Guyancourt, France*
damien.preveraud@adisseo.com

Using the newly harvested grain is like a betting game for the producer of animal feeds who has to deal with unknown contamination. That is why we have developed a new tool dedicated to identifying the mycotoxin risk to allow industry to timely prepare and react to possible feed contamination problems. The model is able to provide a level of risk for fumonisins (FUM), deoxynivalenol (DON) and zearalenone (ZEA) in maize and wheat in agreement with those outlined in the EU legislation. This tool, called Qualimetre, is based on a large dataset collected over the past years including agronomic practices and interactions with climate.
The Qualimetre algorithm has given its prediction (from low to high) before the 2021 wheat and corn harvests in 11 different european countries. Then, after the harvest, we have analyzed by LC-MS/MS the mycotoxins concentrations. For instance, we determined that the risk for DON is high in wheat to be harvested in Russia, Ukraine, Germany, and France. The mycotoxin risk in corn is high for DON and ZEA in Germany, Hungary and Poland, and for FUM in Russia, Italy and Hunagry. Together with the predicted risk, we showed for each country a strong correlation with the predicted risk and the corresponding analytical values.
Having an accurate prediction of the mycotoxins risk of contamination, animal feed producers can thus win extra time for a good preparation and organization of the harvest and optimal grain use.

EFFECTIVENESS OF MYCOTOXIN DEACTIVATOR ON NATURAL MIXED MYCOTOXINS CONTAMINATED FEED FED TO LACTATING DAIRY COWS

LYNCH S.[1], KERNS B.K.[1], SILVA L.H.P.[1], PREVERAUD D.P.[2], DVORSKA J.[2], BROMFMAN G.[3], AVERKIEVA O.[2], WHITEHOUSE N.L.[1]

[1]University of New Hampshire, Dairy Nutrition Research Center, 30 O'Kane Road, Durham, NH 03824 - USA; [2]Adisseo France SAS, Immeuble Antony Parc 2, 10 Place du Général de Gaulle, 92160 Antony, France; [3]Adisseo USA, Inc., One Point Royal Suite 275, 4400 North point Parkway, Alpharetta, GA 30022- USA.

damien.preveraud@adisseo.com

Twenty-four multiparous Holstein cows, 9 cows equipped with ruminal cannulas and 15 non-cannulated cows were used in the study. The duration of the trial was 11 weeks, 1 week to collect covariate data (cows will be feed the control diet) and then 10 weeks with cows on assigned treatments. The proposed study was a replicated randomized block. The experimental treatments were: NC (Basal diet + distillers grains with low mycotoxin load), PC (Basal diet + distillers grains with high mycotoxin load) and AF (PC + 30 g/d Adi-Flow (Adisseo, Belgium)). Milk yield, milk composition, DMI, and feed efficiency were recorded weekly. Blood, rumen juice and urine were collected at -1, 2, 4, 6, 8 and 10 weeks. Degrees of freedom will be calculated using the Kenward-Roger option of MIXED procedure (SAS, 2010). Significance will be noted at $P \leq 0.05$ and trends were noted $P>0.05$ to $P < 0.10$. The challenge groups (PC and AF) has greater content of DON and ZEA mainly compared to NC. Milk fat and protein, linear Somatic Cell Score and milk yield:DMI were negatively affected by the challenge (NC vs PC; $P<0.05$). The supplementation of mycotoxin deactivator aimed to significantly restore these performance and nutritional parameters. At the rumen level, ammonia and some key volatile fatty acids like butyrate, valerate and acetate to propionate ratio were significantly improved in the AF groups after been degraded in presence of mycotoxins (PC). The study showed that dietary mycotoxin deactivator is enable to shift the microbiota and metabolite production to enhance performance of the cows.

LACTOBACILLUS ACIDOPHILUS CIP 76.13 AND L. DELBRUECKII SUBSP. BULGARICUS CIP 101027T AS PROMISING MYCOTOXIN DECONTAMINATING AGENTS

RAGOUBI C.1,2 , QUINTIERI L.1, GRECO D.1 , MEHREZ A.2, MAATOUK I.2 , D'ASCANIO V.1, LANDOULSI A.2, AVANTAGGIATO G.1*

[1]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), G. Amendola 122/O, 70126 Bari, Italy; [2]Risques liés aux stress environnementaux, lutte et prévention, Faculté des Sciences de Bizerte, Zarzouna 7021, Tunisia

giuseppina.avantaggiato@ispa.cnr.it

Mycotoxins are harmful secondary metabolites produced by fungi which contaminate a wide range of food and feed. Lactic acid bacteria (LAB) show a promising potential to degrade or bind mycotoxins thus reducing their absorption at gastrointestinal level. This study was aimed to investigate the ability of Lactobacillus acidophilus CIP 76.13 and L. delbrueckii subsp. bulgaricus CIP 101027T in removing AFB1, OTA, ZEA and DON in culture media. Mycotoxin removal by viable and heat-inactivated cells was evaluated at pH7, 37°C and 24 h of incubation time, in PBS and MRS containing 1 µg/mL of each mycotoxin. Residual mycotoxin content in supernatants and cell pellets was determined by UHPLC-FLD/PDA analytical methods. Mycotoxin reduction values differed depending on liquid media. In PBS, viable cells of these strains reduced, on average, ZEA and DON by 57.4 and 30.0%, respectively. AFB1 and OTA reductions in PBS were negligible, being lower than 15%. In MRS, mean values of ZEA and AFB1 reduction were 28.0 and 32.1%, respectively, while OTA and DON were not reduced. Mycotoxin reductions recorded using heat inactivated cells of each strain, tested in PBS or MRS, were significantly lower than those obtained with viable cells. These results suggests that mycotoxin reduction by bacterial strains may occur by a biotransformation process rather than a binding mechanism. In addition, both strains survived at pH 3, 5 and 7 for 24 h in PBS and exhibited lipolytic and proteolytic activities. This study suggests the potential use and broader application of LABs (CIP 76.13 and CIP 101027 T) for mycotoxin reduction in food and feed industry.

METABOLIZATION OF DEOXYNIVALENOL AFTER ACUTE OR CHRONIC EXPOSURE IN BROILER CHICKENS

RIAHI I. [1*], PEREZ –VENDRELL A.M.[1], MARQUIS V [2], RAMOS A.J.[3], BRUFAU J.[1].

[1]IRTA, Animal Nutrition , Ctra. Reus-El Morell, Km 3.8, 43120 Constanti, Spain; [2]Phileo-Lesaffre Animal Care, 2580 Chemin de Vallesvilles, 31600 Seysses (France); [3]Food Technology Department, University of Lleida. Avda Rovira Roure, 191. 25198 Lleida, Spain.

Insaf.riahi@irta.cat

A comparison study of metabolization of mycotoxin deoxynivalenol in chickens after acute and chronic exposure was performed. Acute exposure was performed via oral and intravenous (IV) application of DON. The chronic exposure was conducted via feeding. Doses of 0.75 and 2.25 mg DON/kg of BW were administrated intravenously and orally to the chickens. The administrated doses corresponded to 5 mg DON/ kg in feed (maximum level recommended by the European Commission in poultry feed) and to 15 mg DON/ kg in feed, respectively. Blood samples were collected and plasma extract samples were prepared for analysis of DON and its metabolites. Chromatographic analysis by LC-MS/MS revealed that the maximal concentration of DON in plasma was 16.6 and 49.7 ng/ml after IV administration of 0.75 and 2.25 mg of DON /kg BW, respectively and DON-S was the major metabolite of DON in broiler chickens blood. Nor DON neither DON-S were found in plasma after oral application and via feeding. For this reason, lyophilized livers of broilers exposed to chronic toxicity with DON in feed, were analyzed to determine DON and DON-S. DON-S was only found in livers for broilers fed 15 mg/kg. The absence of DON and its metabolite DON-S in broilers plasma after oral application or via feeding could be explained by a low absorption and rapid clearance of this mycotoxin. Furthermore, the presence of DON-S both in plasma (after intravenous administration) and in liver (after chronic feeding), indicates this metabolite as the appropriate biomarker of DON exposure in chickens.

BIOLOGICAL DETOXIFICATION OF ZEARALENONE BY BACILLUS SUBTILIS STRAINS

TECHER C.1 , GRECO D. 2 , BRAME A. 1 , D'ASCANIO V. 2 , SANTOVITO E.2 , QUINTIERI L.2 , DE CANDIA S.2 , AVANTAGGIATO G.2

[1]Mixscience, 2/4 Avenue de Ker Lann, CS 17228 35172 BRUZ CEDEX France ; [2]Institute of Sciences of Food Production (CNR-ISPA), Via Amendola 122/O, 70126 Bari (Italy)

clarisse.techer@mixscience.eu.

Zearalenone (ZEA) is a non-steroidal estrogen produced by many Fusarium species in cereals and other plants, and is frequently implicated in adverse health effects and economic losses in livestock production. The aim of the present study was to investigate the efficacy of Bacillus subtilis strains in reducing ZEA and its major metabolites of phase I (α-ZAL, α-ZOL, β-ZAL, β-ZOL, ZAL) in culture media. Batch decontamination experiments were performed using different growth media, temperature, pH, incubation time, toxin concentration, and oxygenation. Desorption/extraction experiments and heat/acid treatment of bacteria were carried out to study the mechanism underlying mycotoxins reduction. Mycotoxin content in culture media and cell pellets was determined by UHPLC-FLD/PDA methods after sample purification with immunoaffinity columns. All tested strains were effective in reducing ZEA and its major metabolites in aerobic and anaerobic conditions, and in growth medium containing the toxin as sole carbon source. Reduction of ZEA and metabolites was >90% in mostly tested conditions. Maximum reduction was obtained in less than 24h. Optimal temperature and pH for toxin reduction were 30°C and pH range at 5-8. Desorption/extraction trials suggested that ZEA removal occurred by a metabolism of the toxin involving enzymatic activities, the latter being deactivated in acid- or heat-treated cells. These activities are intracellular and are not released into the growth medium. Indeed, bacterial cultures and viable cell pellets showed significantly higher ZEA removal efficiencies than supernatants. In conclusion, due to its potential to effectively remove ZEA, B. subtilis could be exploited in food and feed chains to reduce mycotoxin contamination. Further studies will be addressed to identify ZEA metabolites produced by B. subtilis strains during the removal of the toxin and relevant toxicity.

THREE THINGS WE COULD DO BETTER

MILLER D.J. PHD FAIHA
Distinguished Research Professor
Carleton University, Ottawa, Ontario K1S 0M3
DavidMiller@Cunet.Carleton.Ca

I started working on mycotoxins almost four decades ago. At that time, there were several unassailable truths (1) trichothecenes occurred only under cold conditions, (2) chemotypes did not exist and (3) the interaction between crops and the toxins was irrelevant. Some scientists mocked the very idea that aflatoxin was a human carcinogen. I could go on. To that end, I have three thoughts for consideration.

(1) A much better job is needed to assess population exposure to the five important mycotoxins. The time worn method of predicting exposure based on limited crop and food analyses has proven so wanting. For four of the five important toxins, there is a poor understanding of the toxicokinetics in humans including the genetic variability between different populations so important for pharmaceuticals. The use of labelled standards for all the key metabolites and collaborative studies of methods and reference materials are essential. Once this is achieved software that exists for assessing sample number that can be adapted for the important mycotoxins. Much of the data produced now has limited value for public policy.

(2) Geneticists need to stop conflating genotype with genotype. For decades, researchers from Japan, Korea, USA, Canada, Germany and Austria have reported strains that unambiguously produce more than one toxin pattern from stains of DON and T-2 producing fungi. This is not a scientific nicety; our health is not harmed by biosynthetic genes but by toxins. Further, it is important to be aware of the genetic changes underway in several toxigenic fungi.

(3) Make mycotoxin prediction models useful. Since 1978, more than 400 academic studies have been published on mycotoxin prediction in bone fide journals. Nonetheless, there are few commercial systems in operation that have stood the test of time. Models that have been proposed are prone to both false positive and false negatives from the failure to incorporate known biotic and abiotic variables. A number of companies in the USA and Canada run models for forestry and agricultural applications based on the new generation Landsat 9 satellites. This works by incorporating continent scale data to build the algorithms. A breakthrough can only come by combining the expertise of companies whose business to work with these data and scientists with comprehensive expertise in mycotoxins.

RISK ASSESSMENT OF COMBINED EXPOSURE TO MULTIPLE CHEMICALS @ EFSA: FROM GUIDANCE DOCUMENTS TO APPLICATIONS.

DORNE JEAN LOU C.M. [1*] AND HOGSTRAND C.[2]
[1]*Scientific Committee and Emerging Risks Unit European Food Safety Authority, Parma, Italy*
[2]*King's College London , Department of Nutritional Sciences, London, UK*
jean-lou.dorne@efsa.europa.eu

In march 2019, the European Food Safety Authority (EFSA) has been published a guidance document (GD) on harmonised methodologies for the human health (HRA), animal health (ARA) and ecological risk assessment (ERA) of combined exposure to multiple chemicals ("chemical mixtures"). The guidance document focuses on food and feed safety and can be broadened to other regulatory areas and across regulatory silos. A harmonised framework using the principle of tiering has been proposed for both whole mixture and component-based approaches (WMA, CBA) together with stepwise approaches for problem formulation, exposure assessment, hazard assessment, risk characterisation and uncertainty analysis. For a WMA, the mixture is most often assessed using approaches as for a single compound. For the CBA, the default model for assessing combined toxicity is dose addition unless evidence for interactions exist (i.e. synergism, antagonism) and are assessed using a weight of evidence (WoE) approach. Such a WoE analysis allow (semi-) quantification of magnitudes of interactions using either an extra uncertainty factor or a biologically-based model (e.g. toxicokinetic-toxicodynamic model) in the hazard assessment and the risk characterisation steps. For transparency, a summary reporting template has also been proposed. Finally, the GD addresses future perspectives for mixture RA particularly for the integration of historical data, mechanistic alternatives to animal testing (i.e. in silico and biologically-based models). Recent applications are illustrated and include technical reports and peer reviewed publications to implement the GD in HRA, ARA and ERA as well as the MYCHIF collaborative research project dealing with a holistic, innovative and flexible risk assessment modelling approach for mycotoxin mixtures in food and feed.

KNOWLEDGE CENTRE FOR GLOBAL FOOD AND NUTRITION SECURITY (KC-FNS): A NEW EC WEB PORTAL DEDICATED TO MYCOTOXINS. VISION AT GLOBAL LEVEL

ERMOLLI M[1*], REMBOLD F.[1], NEGRE T.[1] AND STROKA J.[2].
[1]European Commission - Joint Research Centre, Directorate D, Sustainable Resources, Ispra (Italy); [2]European Commission - Joint Research Centre, Directorate F, Health - Consumers and Reference Materials, Geel (Belgium).
monica.ermolli@ec.europa.eu

The Knowledge Centre for Global Food Nutrition and Security launched in November 2018 in Brussels supports the collection, exchange and valorization of knowledge on topics of importance to reach food and nutrition security in food insecure countries. One of the topics on which the Knowledge Centre is investing is "Mycotoxins and food security". This theme is deeply connected with food and feed safety and security issues, and the future EU Farm to Fork strategy international dimension, including very obvious implications with health and international trade concerns. Additionally, mycotoxins presence is linked to crops health and climate extreme conditions, aspects which emphasizes its significance. The secretariat of the KC-FNS believes urgent to make progress in the development of the KC web portal. The proposal is to create a dynamic virtual environment for hosting information that could cover several aspects as:

- Share information on climate extremes, post harvested losses and possibly crop health
- Showcase innovative scientific solutions applied to reduce post harvested losses and tackle mycotoxin crop contaminations - Create a system for collecting information about mycotoxin outbreaks.

To achieve these objectives and with the overall goal to support the development of sustainable food systems in food insecure countries, we would like to build strong interaction with existing networks and scientific actors in the domain.

FUTURE REGULATORY DEVELOPMENTS ON MYCOTOXINS IN FEED AND FOOD IN THE EU AND AT CODEX

VERSTRAETE F.

The presence of mycotoxins in feed and food has been increasing in recent years also due to changing weather conditions. Given the high influence of weather conditions on the presence of mycotoxins in feed and food, there is a high year-to-year and geographical variation in the occurrence of mycotoxins in feed and food as the presence cannot be fully controlled by good agricultural practices.
Taking into account the possible animal and public health concern related to the presence of these mycotoxins in feed and food, the mycotoxins are regulated in feed and food to ensure a high level of animal and human health protection. Based on recent scientific advice, the maximum levels in food needs to be strengthened to continue to ensure a high level of human health protection. Also the approach to regulate mycotoxins in feed needs to be strengthened to ensure a high level of animal health protection. These stricter maximum levels in food and stricter regulatory approach in feed are under discussion at technical level, based on the available data in the EFSA database and taking into account the year-to-year variation as reflected in the EFSA occurrence database.
At Codex Alimentarius, discussions are ongoing as regards maximum levels of aflatoxins in peanuts ready-to-eat, aflatoxins and ochratoxin A in spices and aflatoxins in cereals and cereal products. It can also be expected that in the near future discussions on possible maximum levels of ergot alkaloids and T2-HT-2 o, cereals and cereal products will be initiated.

PLATINUM SPONSOR

PATENT CO.®

GOLD SPONSOR

Phileo by Lesaffre

SILVER SPONSOR

NFT New Feed Team

mixscience Innovate for Life

XEMA

r-biopharm®

Biomin®

ADISSEO A Bluestar Company

eurofins | Tecna

BÜHLER

syngenta®

UTOMADE®

AFLA ZERO

OTHER CONFERENCE SPONSOR

LAVIOSA
Advanced Mineral Solutions
ITALY

VICAM®
A Waters Business

AFFILIATED JOURNAL

toxins
an open access journal by MDPI

MEDIA PARTNER

affidia
THE JOURNAL
OF FOOD DIAGNOSTICS

360° SECURITY
TOTAL MYCOTOXINS PROTECTION

PATENT CO.®

PATENT-CO.COM

MINAZEL PLUS, total protection against mycotoxins!

Experts in prevention

min-a-zel® Plus

SafWall

Integrated mycotoxin strategy

- BIOSORPTION
- BARRIER INTEGRITY
- BODY PROTECTION

Phileo by Lesaffre

phileo-lesaffre.com

Phileo by Lesaffre

NFT

New Feed Team

High-quality
products
for the animal
feed industry

SAFE

Adsorpion of traditional and emerging mycotoxins

Here at NFT our aim has always been to purse innovative solutions capable of revolutionizing the market. Emblematic of this philosophy is SAFE - the first line of mycotoxin binders to feature “multi-component” technology, which we have been making since 1993.
Now, NFT has brought its research findings and extensive experience to bear in creating broad spectrum binders meeting organic certification standards, designed to act against both traditional and emerging mycotoxins.

EUROPEAN QUALITY

ITALIAN THINKING

www.nftitalia.com

Multiprotect Y

The widest shield against biotoxins

- Highest safety: Broad spectrum protection
- Bioprotection effect
- Biotoxins Risk Management Program full offer

miXscience
Innovate for Life
contact@mixscience.eu

Avril GROUP

XEMA group manufactures antigens, antibodies and immunoassay kits for medical and veterinary in vitro diagnostics for more than **25** years.

We focus on immunoassays for detection of fungal antigens:

- *Fusarium spp.* ELISA and rapid test
- *Alternaria spp.* ELISA
- *Aspergillus fumigatus* ELISA and rapid test
- *Aspergillus niger* ELISA
- Galactomannan ELISA and rapid test
- *Mucor spp.* ELISA
- Ergot ELISA

Further genuses and species in development – please inquire.

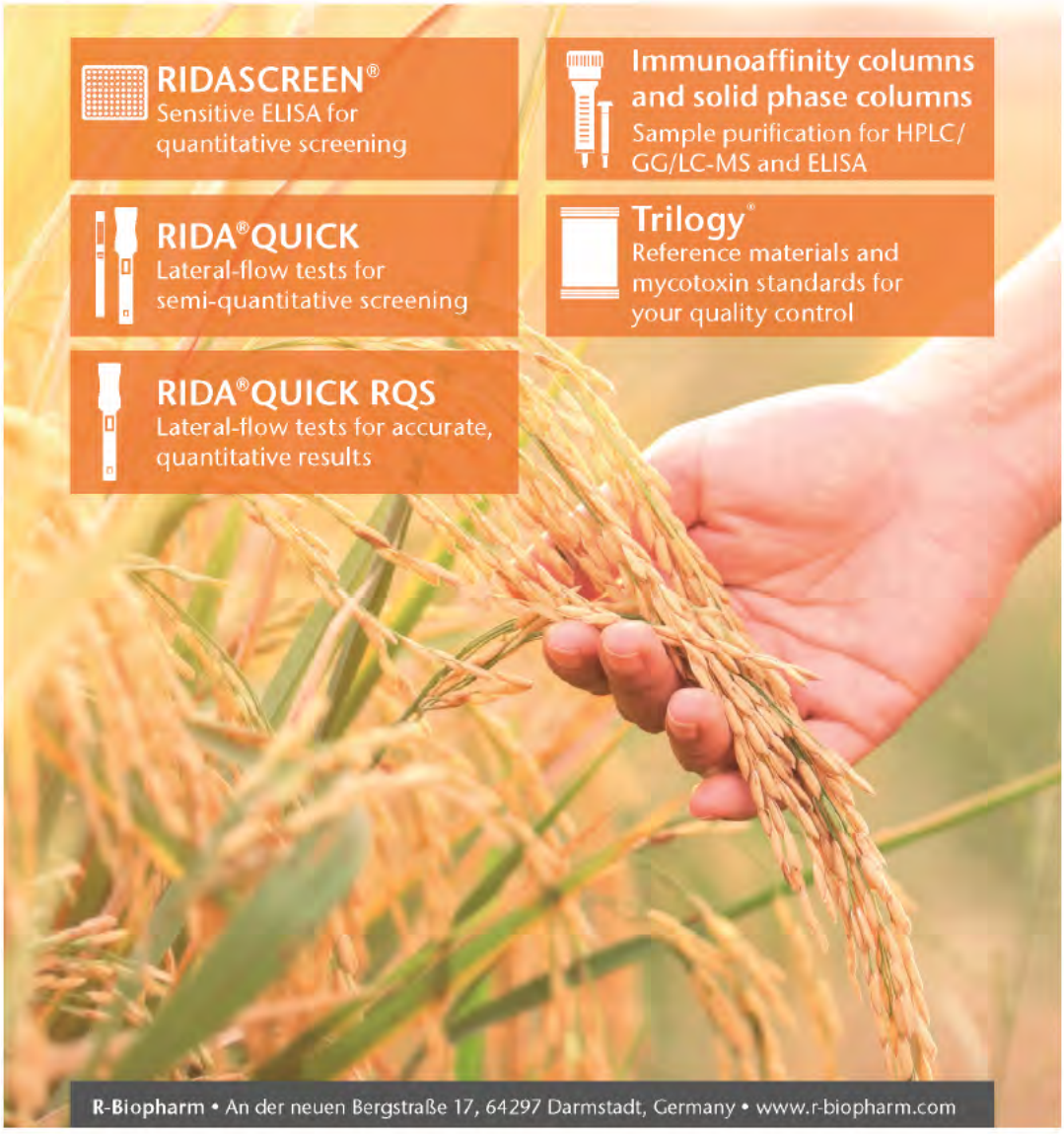
XEMA also manufactures ELISA kits for detection of mycotoxins, antibiotics, allergens and food adulteration markers.

www.xema-medica.com | info@xema-medica.com

R-Biopharm – dedicated to food safety

r-biopharm®

Hands-on quality control for mycotoxins



RIDASCREEN®
Sensitive ELISA for quantitative screening

RIDA®QUICK
Lateral-flow tests for semi-quantitative screening

RIDA®QUICK RQS
Lateral-flow tests for accurate, quantitative results

Immunoaffinity columns and solid phase columns
Sample purification for HPLC/ GG/LC-MS and ELISA

Trilogy®
Reference materials and mycotoxin standards for your quality control

R-Biopharm • An der neuen Bergstraße 17, 64297 Darmstadt, Germany • www.r-biopharm.com

Mycofix® 5.0

MYCOTOXIN RISK MANAGEMENT

Absolute Protection

Powered by science to actively defend against multiple mycotoxins*

With 3 combined strategies

- ADSORPTION
- BIOTRANSFORMATION
- BIOPROTECTION

*Authorized by EU Regulations No 1060/2013, 1016/2013, 1115/2014, 2017/913, 2017/930 and 2018/1568 for the reduction of contamination with fumonisins, aflatoxins and trichothecenes.

mycofix.biomin.net

Naturally ahead

Biomin®

2000-2020

Vent'anni di grandi innovazioni per anticipare le sfide di domani

Quest'anno Syngenta celebra vent'anni di successi.

Vent'anni che ci hanno visto sempre al fianco degli agricoltori per fornire loro gli strumenti necessari per portare cibo di qualità in quantità sulle tavole e per affrontare i cambiamenti continui e le sfide di ogni giorno.

Gli oltre 28.000 dipendenti e ricercatori di Syngenta in tutto il mondo, mentre festeggiano questo importante traguardo, guardano già al futuro, accelerando il passo dell'innovazione per rispondere sempre più velocemente alle necessità dell'agricoltura.

Bringing plant potential to life

syngenta®

®

AFLA ZERO

The circular economy solution to aflatoxin

March 2020

People at risk:
4.5 billion people

First pilot plant for use in animal feed (Kenya):
June 2020

First commercial decontamination facility for human food:
Q1 2021

120 MT commercial plant

Contact

Chris Mimm
email: chris@aflazero.com
phone: +254 790 499 245

Aflazero, Ltd.
www.aflazero.com

Problem:

- The Food and Agriculture Organization of the United Nations estimates that **25 percent of world food crops are affected by aflatoxin**, which presents a significant threat to the health, economy and food security of most developing world countries.
- The Center for Disease Control estimates **that over 4.5 billion people in the developing world are exposed to aflatoxins.**

Solution:

- **Aflazero was incorporated in 2017** to commercialize the first viable technology to remove aflatoxin from grains, nuts and pulses.
- This technology is based on **10 years of research and development**, a portion of which was funded by the European Union.
- The initial success in the lab (removing over 95% of aflatoxin from contaminated grains) is now being **scaled up for country-level applications**.

Aflazero's technology is a market-based solution that benefits consumers, farmers, governments, commodity traders and food processors, and development organizations:

◊ **Consumers:** Anyone in Africa who consumes staple food products is exposed to aflatoxin levels that exceed acceptable health standards.

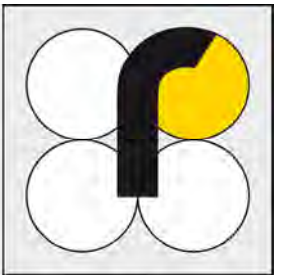
◊ **Farmers:** Aflazero enables food commodity buyers, processors and traders to buy crops from farmers that would otherwise be rejected due to aflatoxin.

◊ **Governments:** Aflazero is the only solution available for decontaminating national strategic grain reserves (other than incineration at 800 degrees Celsius).

◊ **Commodity traders and food processing companies:** Aflatoxin-contaminated commodities are often rejected by food processors and traders, as well as by international regulatory agencies. Aflazero enables these companies to buy crops from African producers for export to higher value markets such as Europe.

◊ **Development organizations:** The United Nations delivers staple crops to millions of beneficiaries each year. Aflazero can provide decontamination at the aggregation warehouses, rendering the crops safe for delivery to local communities.

r-biopharm®



ADISSEO

A Bluestar Company


eurofins
Tecna


UTOMADE®

P.le Aldo Moro 7
00185 Roma