ICFM

International Commission on Food Mycology

Conference 2019

Food- and Airborne Fungi – Challenges for Food Safety and Supply

Programme and Abstracts

Freising - Germany, 3-5 June, 2019

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Food- and Airborne Fungi – Challenges for Food Safety and Supply

Freising, Germany - 3-5 June, 2019

INTERNATIONAL COMMISSION ON FOOD MYCOLOGY

The commission is a COMCOF (Commissions, Committees and Federations) of the International Union of Microbiological Societies (IUMS) and established in 1990.

The aims of the Commission are:

- to improve and standardise methods for isolation, enumeration and identification of fungi in foods;
- to promote studies of the mycological ecology of foods and commodities;
- to interact with regulatory bodies, both national and international, concerning standards for mycological quality in foods and commodities;
- to support regional initiatives in this area. The Commission further aims to extend understanding of the principles and methodology of food mycology in the scientific community by publishing its findings, and by sponsoring meetings, specialist workshops, courses and sessions dealing with aspects of its work

Venue:
Freising is a 50.000 citizen community situated 40 km north east from the city of Munich, which can be reached by train in 20 min. Munich Airport is close by with a direct bus connection.

The city is well known for its rich ecclesiastic history as well as for its importance as a centre of food science and technology as well as beer brewing. The Freising Cathedral (built 1205) it is one of the two home churches to the archbishop of Munich and Freising, one of which was Cardinal Josef Ratzinger, who was elected pope Benedikt XVI in 2005. The workshop will take place at the Pallotti Haus, Pallottinerstraße 2, 85354 Freising (phone +49 08161 9689 0 email freising@pallottiner.org)

The ninth International Foodmycology workshop is organized by

Ludwig Niessen
Technical University of Munich, Chair for Technical Microbiology, Gregor-Mendel-Str. 4, 85354 Freising, Germany

and

Rob Samson
Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

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PROGRAMME ICFM 2019

Sunday, 2 June 2016

16.00-18.00 Registration

18.00 Get together at "Keller-Stüberl" bar at Pallotti-Haus (drinks and finger food)

Monday, 3 June 2019

08.00-09.00 Registration continued

09.00 Ludwig Niessen: Opening of the workshop

09.00-09.30 John I. Pitt: A short history of food mycology

Session 1: Heterogeneity of food borne fungi (Session chair Ludwig Niessen)

09.30-09.55 Jan Dijksterhuis: Heterogeneity of stress-resistance of fungal conidia

09.55-10.20 Maarten Punt: Heterogeneity in conidia of the spoilage fungi *Penicillium roqueforti*

10.20-10.45 Tom van den Brule: Strain variability in three strains of the food spoilage fungus *Paecilomyces variotii*

10.45-11.10 Sjoerd Seekles: Heterogeneity in food spoiler *Aspergillus niger*, the effect of spore age and cultivation conditions on conidial heat resistance

11.10-11.30 Coffee break

Session 2: Biodiversity and taxonomy of food borne fungi (Session chair Robert A. Samson)

11.30-11.55 Évelin Wigmann: MALDI-TOF MS based differentiation and identification of 49 species within the *Fusarium fujikuroi* species complex

11.55-12.20 Mohamed Fathi Abdallah: Biodiversity of mycotoxigenic fungi and their secondary metabolites isolated from sugarcane juice

12.30-13.30 Lunch at Pallotti-Haus

13.30-13.55 Miloslava Kavkova: The evidence of common species of the opportunistic fungal pathogens in dairies and bioptic samples

13.55-14.20 Jos Houbraken: Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins and other mycotoxins

Session 3: Influence of environmental factors on food borne fungi (Session chair Naresh Magan)

14.20-14.45 Abigail Snyder: Association of fungal genera from spoiled processed foods with physicochemical food properties and processing conditions

14.45-15.10 Esther Garcia Cela: Modelling the effect of temperature and water activity on the growth boundaries of *Fusarium graminearum* in stored wheat

15.10-15.40 Coffee break

15.40-16.05 Oceane Savary: Comparative study of *Bisifusarium* spp. growth patterns by laser nephelometry automated microplate system

16.05-16.30 Andrea Patriarca: From field to process: how storage selects toxigenic *Alternaria* spp. in apples

16.30-16.55 Frank Segers: The physiology of food-borne fungi and organic acid production in response to environmental conditions

17.00-18.30 Poster session (meet presenters at their poster)

19.00 Dinner at Pallotti-Haus

Tuesday, 4 June 2019

Session 4 Control and biocontrol of food- and airborne fungi, mycotoxin producers and heat resistant molds (Session chair Emilia Rico-Muñoz)

08.30-08.55 Sofia Chulze: Biocontrol of *Fusarium graminearum* and deoxynivalenol accumulation during the micromalting process

08.55-09:20 Alejandro Gimeno: Suppressing *Fusarium graminearum* and mycotoxins by application of microbial biopesticides on infested crop residues

09.20-09.45 Dimitrios Drakopoulos: Chasing *Fusarium graminearum* throughout its entire life cycle with botanicals: An in vitro approach

09.45-10.10 Markus Schmidt-Heydt: Zero Aflatoxin – Development and implementation of antifungal strategies to increase food safety in sub-saharan Africa

10.15-10.35 Coffee break

10.35-11.00 Naresh Magan: Fungal diversity and biocontrol of afaltoxins in GM- and non-GM maize cultivars

11.00-11.25 Juliane Lane P. dos Santos: Controlling the spoilage of fruit purees by heat-resistant moulds (HRMs): Diversity of HRMs during processing and inhibition of their growth during storage

11.25-11.50 Emmanuel Coton: Potential of antifungal lactic acid bacteria combinations as bioprotective cultures in pilot scale dairy products

11.50-12.15 Vasilis Valdramidis: Design and up-scaling of filters coated with zinc oxide nanoparticles in pear warehouses

12.30-13.30 Lunch break at Pallotti-Haus

Session 5: Mycotoxinogenic fungi and mycotoxins (Session chair Rolf Geisen)

13.30-13.55 Brigitte Andersen: Mycotoxins in animal feed

13.55-14.20 Jens C. Frisvad: Comparative mycology and chemistry of apple and citrus fungi

14.20-14.45 Marta H. Taniwaki: A „snapshot“ of Biodiversity of *Aspergillus* section *Flavi* in Brazilian foodstuffs

14.45-15.10 Maria Laura Ramirez: Fumonisin occurrence in wheat and wheat by products

15.10-15.35 Xenia Pascari: Transfer of deoxynivalenol and deoxynivalenol-3-glycoside from barley to boiled wort

15.40-16.00 Coffee break

16.00-16.25 Monika Coton: Production and migration of ochratoxin A and citrinin in *Penicillium verrucosum* molded Comté cheese

16.25-16.50 Endang S. Rahayu: Quality control of mycotoxin producing fungi contamination in cocoa beans and its product: Case study in Indonesia

17.30-19.00 ICFM committee meeting (closed meeting)

19.30 Foot walk to "Hofbrauhaus-Keller Freising"

20.00 Dinner at "Hofbrauhaus-Keller Freising", traditional Bavarian restaurant/beer gadgen, downtown Freising

Wednesday, 5 June, 2019

Session 6: Sanitation of food spoilage fungi (Session chair Vasilis Valdramidis)

08.30-08.55 Marina Venturini Copetti: Adequate sanitizers for controlling food spoilage fungi

08.55-09.20 Elettra Berni: Sanitization by hydrogen peroxide and peracetic acid: are heat resistant moulds the best choice for bio-validations of food packaging and machineries?

09.20-09.45 Emilia Rico-Muñoz: Mold spoilage of heat-processed/heat-filled baverages: when everything goes wrong!

Session 7: Fungal genetics and genomics (Session chair Jos Houbraken)

09.50-10.15 Alicia Rodriguez: Changes in the expression of a gene involved in cell wall integrity are related to mycotoxin production in filamentous fungi in food

10.15-10.40 Rolf Geisen: Regulation of mycotoxin biosynthesis by *Penicillium expansum* as an important determinant of the colonization capacity

10.40-11.10 Coffee break

11.10-11.35 Gemma Castella Gómez: Genomic diversity in ochratoxigenic and non- ochratoxigenic strains of *Aspergillus carbonarius*

11.35-12.00 Ludwig Niessen: PCR-based distinction of high and low toxic S-type strains in *Stachybotrys chartarum*

12.00-12.30 Final discussions, closing of the conference

12.30 Lunch at Pallotti-Haus, Checkout

POSTERS

1. **Shaimaa Abdelmohsen**: Solute and matric potential stress and *Penicillium verrucosum*: impacts on growth, gene expression and ochratoxin A production
2. **Diyaa Al-Jaza**: Effect of SO2 (NaMBS) on in situ populations and AFB1 production of *A. flavus* isolated from chillies
3. **Rachel Bertoldo**: *Aspergillus* section *Nigri* in onions bulbs – Occurrence, identification and production of fumonisin B2 and ochratoxin A
4. **F. Javier Cabañes Sáen**z: *Aspergillus welwitschiae* isolated from grapes produce ochratoxin A
5. **Carla Cervini**: Impact of interacting climate change factors on the ecophysiology, toxin gene expression and ochratoxin A production by *Aspergillus carbonarius*
6. **Emmanuel Coton**: Identification and quantification of metabolites produced by antifungal bioprotective cultures in dairy products
7. **Liliana de Oliveira Rocha:** Diversity of *Fusarium* spp. and detection of seventeen mycotoxins in Brazilian brewing barley
8. **Jan Dijksterhuis:** The preservative propionic acid differentially affects survival of conidia damages germ tubes of feed spoilage fungi
9. **Jan Dijksterhuis:** Inactivation of stress-resistant ascospores of Eurotiales by industrial sanitizers
10. **Jan Dijksterhuis**: The shelf life of daily fresh bread is markedly enhanced after short, intense UV treatments
11. **Soňa Felšociová**: Occurrence of *Penicillium* species in healthy grapes for wine production
12. **Lisa M. Frisch**: Development and optimization of a group-specific loop-mediated isothermal amplification (LAMP) assay for the detection of patulin-producing *Penicillium* species
13. **Ana Isabel Galván Romero**: Mycotoxins and toxigenic fungi in dried figs from different farming systems
14. **Jos Houbraken**: Recent developments in the (infrageneric) classification of *Aspergillus* and *Penicillium*
15. **Hyun Jung Lee:** Reduction of Ochratoxin A in Oats during Roasting with Reducing Sugars
16. **Ligia Manoel Martins**: Influence of peanut harvest date on *Aspergillus* section *Flavi* infection and fatty acid composition
17. **Alberto Martin**: Antifungal capacity of phenolic acids against fungal postharvest pathogens
18. **Zuzana Maŝková**: Occurrence of *Ascosphaera apis* in fermented bee pollen
19. **Angel Medina Vaya**: Transcriptomic and metabolomic shifts on *Aspergillus flavus* during maize storage at different climate change environmental conditions
20. **Almudena V. Merchán**: Screening for detection of autochthonous yeast with antagonist activity against spoil molds from raw ewe's milk cheese
21. **Nicolas Nguyen Van Long**: Intraspecific variability in cardinal growth temperatures and water activities within a large diversity of *Penicillium roqueforti* strains
22. **Monica Olsen**: Is it safe to scrape the mould off your food and eat what's underneath it? The example of growth and mycotoxin production of *Penicillium expansum* on apple jam and *P. verrucosum* on crème fraiche at 4, 8 and 15°C
23. **Laís T. Ono:** Fungi and toxins in cassava: from the field to consumer
24. **Gabriela A. Pena**: Effect of ZnO-Nanoparticles on *Aspergillus flavus* and *Fusarium proliferatum* growth on maize grains under environmental interacting conditions
25. **Adriana R. Persson da Silva**: Toxigenic fungi and mycotoxins in black pepper
26. **Eva-Maria Priesterjahn**: Development of new avoidance strategies for aflatoxin contamination in maize grains in the framework of AflaZ, a new project to control aflatoxins in food
27. **Alicia Rodríguez**: Volatile organic compounds produced by yeasts inhibit mycotoxin production by postharvest pathogenic moulds

28. **Cindy J. Romero:** Mycobiota and mycotoxin occurrence in chickpea produced in Argentina
29. **Davide Sardella**: Predictive models for quantitative assessment of antifungal activity of nanoparticles (NPs)
30. **Nicoletta Scaramuzza**: Heat-resistance of *Humicola fuscoatra* and *Talaromyces wortmannii*, two fungal species contaminating industrial packaging materials
31. **Alexandra Schamann:** Inhibition of the aflatoxin biosynthesis of *A. flavus* by arginine
32. **Josué J. Silva**: Analysis of genetic diversity in the *Aspergillus niger* clade
33. **Dana Tančinová**: The effect of selected essential oils on the growth of *Botrytis cinerea*
34. **Tran Trang:** Difference in local pre-harvest practices linked with the occurrence of *Fusarium* species in maize grown by two farmer groups in Vietnam's central highlands
35. **Susanne Vogelgsang**: An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins

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A SHORT HISTORY OF FOOD MYCOLOGY

John I. Pitt*

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"Food mycology" was first used to describe food manufacture with the aid of fungi by the US microbiologist Dr Larry Beuchat in 1985, with the publication of his book "Food and Beverage Mycology". The term "food mycology" has survived, but over time has become used for the study of the role of fungi in food spoilage and the health of human and animals. Food mycology as we now know it was borne out of the intersection of several existing disciplines: food microbiology – the use of Petri dishes, selective media and dilution counting; seed pathology, which provided direct plating; fungal taxonomy, essential for naming and classifying foodborne fungi; plant pathology as some food spoilage fungi are preharvest pathogens of fruits and vegetables; the fundamental study of water relations, as many foodborne fungi grow under low water activity conditions; and organic chemistry, essential for the study of secondary metabolites, some of which are now known to be mycotoxins. International collaboration commenced with the organisation of a meeting called "Standardisation of Methods for the Mycological Examination of Foods" in Boston in 1984. After a second workshop in Baarn in 1990, the organisation morphed into the International Commission on Food Mycology, which as we can all see is today still actively promoting food mycology research. This paper will describe some of the milestones that occurred along the way, and some of the people who were instrumental in the development of food mycology as a discipline in its own right.

SESSION 1: HETEROGENEITY OF FOOD BORNE FUNGI

Heterogeneity of stress-resistance of fungal conidia

Jan Dijksterhuis[1,2]

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The function of spores is to disperse fungi to new areas and to get them through difficult periods. This also makes them important vehicles for food contamination. This contribution aims to explore the connection between prevention of fungal food spoilage to spore biology and modeling studies. It introduces the fungal spore as a vehicle of survival and distribution and discusses its variety and stress resistance. Spores (conidia) are not static particles; they show maturation and change in time. How long spores survive in the food production environment is not known, but the presence of water is important. At the limits of growth, spore germination, which is also highly responsive on environmental cues, becomes unpredictable and shelf life becomes variable. To prevent spoilage of processed foods and drinks, industry often challenges their products based on the worst-case spoilage scenario. For this, knowledge of the range of stress resistance of a population of spores is very important. The variation between fungal strains, thus intraspecific variation, with respect to stress resistance of spores is another scarcely studied area.

Heterogeneity in conidia of the spoilage fungi *Penicillium roqueforti*

Maarten Punt[1,2*], Tom van den Brule[1,3], Wieke R. Teertstra[1,2], Robin A. Ohm[1,2], Jan Dijksterhuis[1,3], Heidy den Besten[1,4] and Han A. B. Wösten[1,2]

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At the moment, 25% of the food is spoiled, a significant part due to fungal contamination. Fungal food spoilage can be found in all food categories. For instance Penicillium roqueforti which is a spoilage fungus of grain and dairy products. Fungal food spoilage often starts with a contamination with spores. These reproductive structures are abundant in the environment. Experimental data strongly indicate the existence of subpopulations of spores with different levels of resistance to preservation methods. The aim of this project is to study the extent of this heterogeneity and to study the underlying mechanisms using spores of Penicillium roqueforti as model system. The role of environmental conditions, and the developmental state of the mycelium and spores are discussed.

Strain variability in three strains of the food spoilage fungus *Paecilomyces variotii*

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Fungal food spoilage often begins with contamination by spores. To prevent spoilage of processed foods and drinks, industry often challenges their products with the worst-case spoilage scenario. Intraspecific variation of food spoilage fungi should be taken into account when defining the worst case presented by fungal spores. In this study, we explored strain variability of the important food spoilage fungus

and heat-resistant mould *Paecilomyces variotii*. In total, 108 strains isolated from various locations and different environments were screened for conidial heat resistance. To discover the limits of this resistance, we quantified D-values for two sensitive and one resistant strain. Further in-depth analysis revealed that the three selected strains showed differences in morphological characteristics, spore size distributions and compatible solute compositions. To our knowledge, strain DTO 217-A2 produced the most heat-resistant conidia ever measured. Altogether, we found intraspecific heterogeneity between the three strains in all experiments presented in our work. This work emphasizes the importance of studying strain variability in predictive food mycology.

Heterogeneity in food spoiler *Aspergillus niger*, the effect of spore age and cultivation conditions on conidial heat resistance

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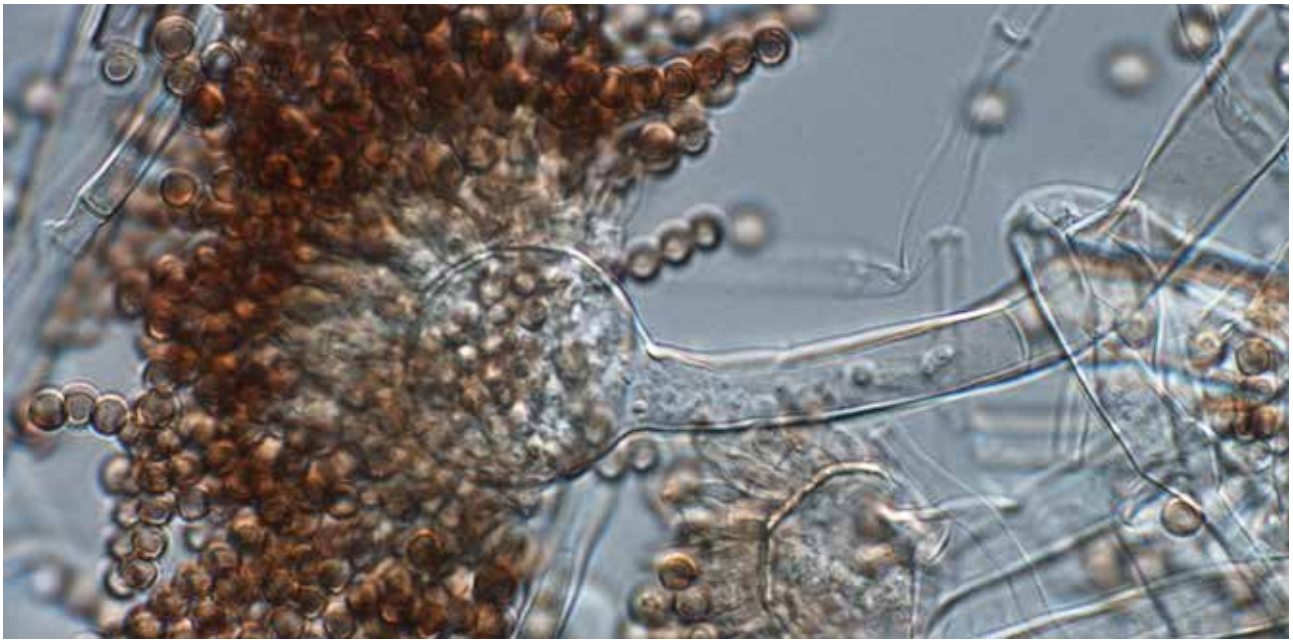
Food spoilage has been a major problem for the food industry. At the moment, a significant part of food spoilage and food waste can be attributed to fungal contamination and spoilage. Food preservation methods like sterilization and salt addition reduce spoilage enormously. However, consumers prefer minimal processing of food to maintain taste and nutritional composition, which leads to increased risk of fungal spoilage. Therefore, fungal food spoilage research is needed in order to promote new and enhanced food processing protocols. Food production needs to increase by 70% to feed the world population in 2050. Reducing post-harvest food spoilage could significantly contribute to this challenge. At the moment, 25% of the food is spoiled, a significant part due to fungal contamination. Fungal food spoilage occurs in all food categories. For instance, *Aspergillus niger* is a known food spoiler of fresh fruits and vegetables. Fungal food spoilage starts with contamination of food products with conidia. These asexual reproduction structures are abundant in the environment, making contamination inevitable. Minimal processing of these contaminated food products proves to be challenging. Experimental data strongly indicates the existence of subpopulations of conidia with different levels of resistance to preservation methods. The aim of this project is to study the extent of this heterogeneity and to study the underlying mechanisms using fungal model systems. In this work, we will elaborate on this heterogeneity by investigating the impact of environmental conditions (differences in growth conditions), and the developmental state on heat stress resistance of *Aspergillus niger* conidia.



SESSION 2: BIODIVERSITY AND TAXONOMY OF FOOD BORNE FUNGI

MALDI-TOF MS based differentiation and identification of 49 species within the *Fusarium fujikuroi* species complex

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Fusarium spp. affect the quantity and quality of economically important crops worldwide. Several species belonging to the *Fusarium fujikuroi* species complex (FFSC, formerly Gibberella fujikuroi species complex) are pathogens on staple crops such as rice, maize, wheat, barley and sorghum. Besides quality and productivity reduction of crops, fungal spoilage can affect human and animal health due to their production of fumonisins and other mycotoxins. Since conventional and sequence-based identification techniques are time consuming, expensive and need expertise in fungal morphology, there is need for the development of alternative methods for accurate species level identification. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis of sub-proteomes has been applied as a promising tool for the discrimination of closely related species in various groups of organisms, mainly focusing on medically relevant microorganisms. In order to reliably discriminate and identify species in the FFSC, we constructed a MALDI-TOF MS spectra database that included spectra from 49 of the currently 61 species described in the FFSC. The database was an in-house supplement to the Bruker Daltonik database for microorganisms. The Bruker standard sample preparation protocol for pure culture fungal mycelia was optimized to achieve a higher discriminatory power. The 49 FFCS species were represented by reference spectra of 87 strains, each of which was confirmed by TEF1α sequence analysis. The database was validated through the analysis of another 80 FFSC strains that were external to the database. The overall rate of correct identifications at the species level was 94.6 %. Identification levels below 100 % were only found in F. musae (17 %), F. ananatum (67 %), F. circinatum (67 %), F. ramigenum (73 %), F. bulbicola (80 %) and F. fractiflexum (83 %). Our data show that MALDI-TOF MS Biotyping provides a highly efficient technology for the differentiation and identification of species within the FFSC. The new method provides a time efficient alternative to the identification based on morphology or the Multi Locus Sequencing Technology (MLST).

Diversity of mycotoxigenic fungi and their secondary metabolites in sugarcane juice: the forgotten beverage

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Sugarcane juice is a traditional beverage consumed on a daily basis, by millions of people, in different countries such as Brazil, Egypt, India and Pakistan. Detection of mycotoxigenic fungi in sugarcane fields has been documented. However, very little is known about mycotoxin contamination in the other sugarcane-based products, especially sugarcane juice (Abdallah et al. 2016). The current study investigated the co-contamination of several mycotoxins (AFB1, AFB2, AFG1, AFG2, DAS, DON, FB1, FB2, FB3, OTA, STERIG, T-2, and ZEN). Further, a polyphasic approach was applied in order to identify and classify the isolated fungal species (2). Mycotoxin contamination results have been used to estimate the dietary exposure of the Egyptian population to mycotoxins through juice consumption. Samples (n=89) were randomly collected from local vendors and juice shops during two seasons (summer 2016 and winter 2017) from Assiut City, Upper Egypt. Extraction and quantification of mycotoxins were performed by liquid-liquid extraction followed by a validated UPLC-MS/MS analytical method. Morphological and molecular identification of mycotoxigenic fungi and other microorganisms was

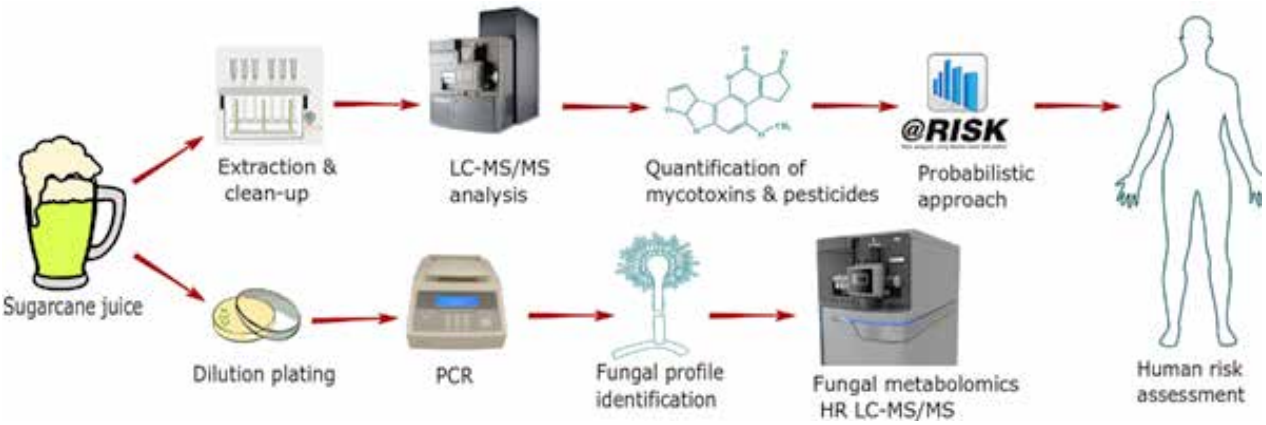

Figure 1: Proposed workflow to determine the commonly occurring mycotoxins in sugarcane juice. Food Frequency Questionnaires will be used to collect data for juice consumption in Egypt that will be used to conduct risk assessment studies. An untargeted approach will be used to screen and identify the unknown fungal secondary metabolites.

performed according to Samson et al.(2010). Dereplication strategy was also applied to unravel the diversity of the produced secondary metabolites using an in-house screening library with a fast DDA UHPLC–TOF-MS profiling method that can screen for hundreds of mycotoxins and other metabolites. Risk assessment of mycotoxins using probabilistic and deterministic approaches at various scenarios for adult male and female Egyptian juice consumers was performed. So far the obtained results for the targeted analysis exhibit the contamination of sugarcane juice samples with AFB1 and FB1 mycotoxins in 63% (n=56) of the samples. Juice collected during winter had a contamination range of 0.1-3 µg.L–1 for AFB1 and 4-58 µg.L–1 for FB1, while samples from the summer season had a contamination range of 0.3-1.3 µg.L–1 for AFB1 only. Furthermore, the risk assessment using probabilistic and deterministic approaches for adult male and female Egyptian juice consumers pinpointed a remarkable difference in levels of exposure to mycotoxins between the two seasons and between males & females. Other results will be presented during the workshop.

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The evidence of common species of the opportunistic fungal pathogens in dairies and bioptic samples

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The opportunistic fungal pathogens (OFP) are represented by fungal species that follow a wide spectrum of life strategies and inhabit various ecological niches. The OFP in dairy operations causes severe problems in spite of massive preventive measures. Contamination of products results in economic loss but also pose a health risk for workers and consumers. The objectives of this study were 1. To survey the occurrence of OFP in the environment and in products in HCCP dairies and family farm dairies and 2. To evaluate the OFP as causal agents of mycoses. The fungal contaminants were isolated and identified from the environment concerning their adaptability to the extreme conditions in dairy operations such as salt bath, pasteurization, temperature, sanitation. The microbial and fungal diversity of milk products from pasteurised and non-pasteurised milk were compared. The fungal isolates were analysed by using molecular methods and sequencing combined with classical microbiological methods. The anonymous bioptic samples were analysed based on the type of immunodeficiency and disease etiology to obtain the current status about the pathogenicity of OFP for immunocompromised individuals. NGS methods and DNA barcoding were further analyzed these samples to assign individual OFPs to the relevant pathological findings. The results showed that many OFP identified in pulmonary, dermatological and

colon bioptic samples such as *Aspergillus nigricans*, *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp. are also representatives of dairy contaminants. These species have a high adaptability and resistance to the extreme environment, antibiotics, and sanitation. The fungal diversity between HCCP dairies and farm dairies was found to be significantly different. The prevalence of *Penicillium*-species and yeast contaminants was found in farm samples whereas the occurrence of OFP contaminants resistant to sanitary chemicals was noted in HCCP dairies first at all.

Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins and other mycotoxins

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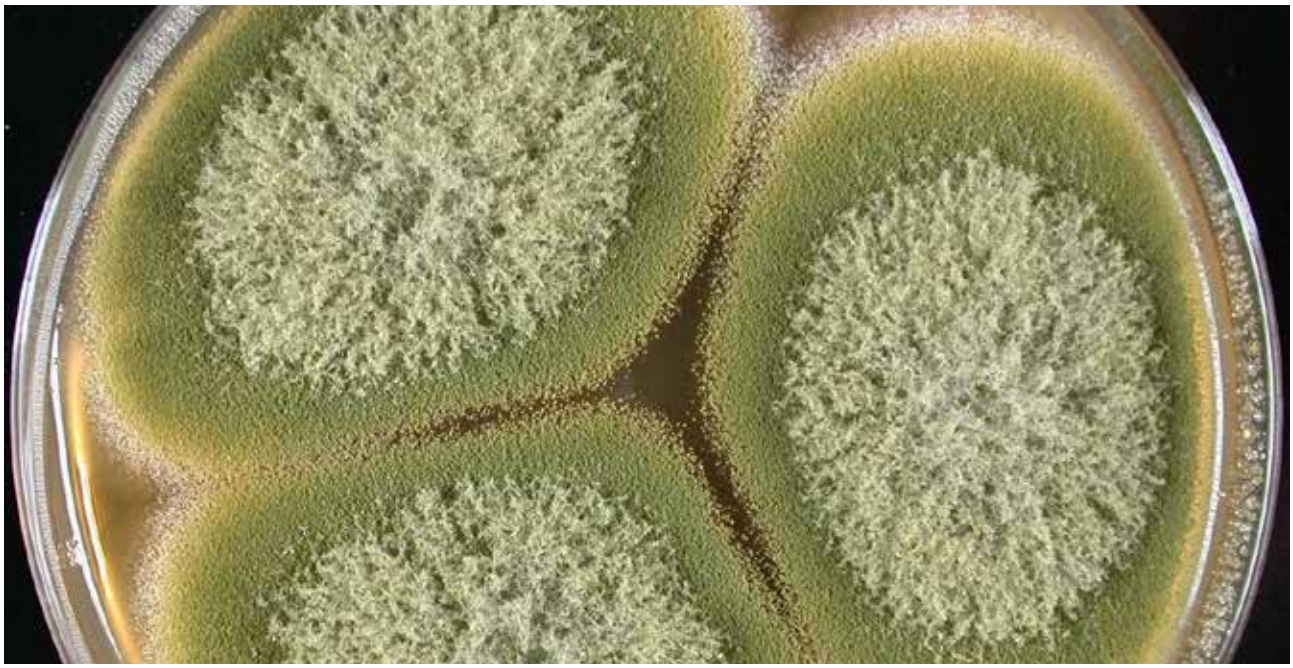
Aflatoxins and ochratoxins are potent mycotoxins and can occur in diverse agricultural commodities. These mycotoxins are reported in several (unrelated) species and genera, and within the Aspergilli, these mycotoxins are mainly produced by members belonging to *Aspergillus* sections *Flavi* (aflatoxins), *Circumdati* and *Nigri* (ochratoxins). Some species used in food fermentation processes appear to be closely related to producers of these mycotoxins. For example, *Aspergillus oryzae* and *A. sojae* are typical industrial moulds that are unable to produce aflatoxins, while their wild counterparts (*A. flavus*, *A. parasiticus*) do. The presence of these fungi in natural fermentations can therefore be of concern. Recent data indicate that several species assigned to these sections cannot be distinguished based on morphological features alone. Since the introduction of molecular techniques, the taxonomy of these sections is studied various times and several new species were described in these sections. We re-evaluated the taxonomy of section *Flavi* based on a combination of sequence data (*BenA*, *Cmd* and/or *RPB2*), phenotypic characters and extrolite (incl. mycotoxins) patterns. In this study, we give an overview of currently accepted species and show that section *Flavi* harbours more species than currently described. Data on extrolite profiles including mycotoxins (aflatoxins) produced and phenotypic characters of section *Flavi* species will be presented. This study shows that section *Flavi* species are potent aflatoxin producers and correct species identification will give insight in potential food safety risks.



SESSION 3: INFLUENCE OF ENVIRONMENTAL FACTORS ON FOOD BORNE FUNGI

Association of fungal genera from spoiled processed foods with physicochemical food properties and processing conditions

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The processing conditions and physiochemical properties used in food manufacturing create niches which support the growth of a limited number of spoilage fungi. This study was designed to evaluate the influence of intrinsic and extrinsic food product variables on the identity of specific spoilage fungi isolated from commercially produced foods. The spoilage etiology was identified in 127 products through ITS region sequencing. The prevalence and diversity of the identified spoilage fungi were evaluated in relationship to product-specific attributes using various descriptive statistics and a bipartite network analysis. Additionally, recursive partitioning was used to generate a classification tree with the outcomes, genera of the spoilage isolates, divided into increasingly homogenous subgroups. All of the isolated fungi belonged to the Ascomycete phylum, except four mucoralian isolates and the basidiomycete *Rhodotorula*. The occurrence of filamentous fungi repeatedly isolated ranged from 2% (*Phoma* spp.) to 18% (*Penicillium* spp.). In order of decreasing contribution to subgroup homogeneity, the split rules for the classification tree were based on process, water activity, food matrix category, and pH. Fungal genera representation in the terminal nodes indicated that production failures, in addition to product-specific attributes, were responsible for determination of the most probable specific spoilage organism.

Modelling the effect of temperature and water activity on the growth boundaries of *Fusarium graminearum* in stored wheat

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The aim of this study was to develop suitable validated models to predict the growth of *Fusarium graminearum* in wheat. For this purpose, two *F. graminearum* strains from wheat were inoculated, in food simulated media (agar wheat medium 3%) adjusted to five different water activities (a_W) 0.88, 0.91, 0.94, 0.97 and 0.99. The cultures were incubated at 6.5, 8, 10, 15, 20, 25, 30 and 35°C and colony growth measured daily for 30 days. The primary model of Baranyi and Roberts (1994) was fitted to the experimental data and the maximum growth rate (μ_{max}, mm/day) and lag time before growth (λ, day) were estimated. In general, a lag-linear curve with some exceptions at 6°C/0.97 a_W and 15-20°C/0.91 a_W was observed. The differences between the two strains tested for μ_{max} and λ were not significant ($p>0.05$). No growth was observed at 0.88 a_w and 35°C. Overall μ_{max} increased with increasing a_w and temperatures up to 25°C (the optimal growth temperature).

In parallel, a logistic regression model including a_W, temperature and time as factors was fitted to the growth/no-growth data. Taking into account the full matrix, the model predicted correctly 95% of the cases in both strains. Probabilistic growth model validation was carried out using two independent data sets of *F. graminearum* on gamma irradiated wheat kernels free of other contaminating fungi. The first set of data was generated at the boundaries of the domain of the model. There was an agreement of the 64% in the concordance matrix. There was an overall discordance for 10 a_w/temperature combinations. However, most of the erroneously predicted cases were on the safer side (false-positives), except at 35°C and $a_W > 0.92$. Better predictions were observed with the second set of data taken from the literature

(Ramirez et al., 2006) temperature (5, 15, 25 and 30 °C where prediction for two strains of the fungus examined showed a 83% concordance. There was only one false negative at 0.90 a_W / 15°C.

Probabilities of growth for *F. graminearum* over 0.80 were predicted in the range 0.90-0.95 a_w at 16-34°C after 30 days. Thus, to avoid type B trichothecenes and zearalenone contamination of wheat an a_w < 0.89 a_W should be maintained, and temperatures in the range 18-31°C should be avoided (P<0.5). On the other hand, probabilities <0.10 were obtained at temperatures <15°C as long as a_W was below 0.89 a_w.

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Comparative study of *Bisifusarium* spp. growth patterns by laser nephelometry automated microplate system

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The genus *Bisifusarium* includes some filamentous fungi previously described as *Fusarium*. Species belonging to this genus have been described in various environments with different characteristics. *Bisifusarium nectrioides*, *B. penzigii, B. delphinoides* and *B. biseptatum* have been associated to natural environments (e.g. plants), *B. lunatum* and *B. dimerum* have been described as human pathogens while *B. domesticum* has only been described in the cheese environment to date. The link between these species and their habitats raises questions in terms of how these fungal species adapt to their environment and how different factors (biotic or abiotic) can influence fungal growth.

In this study, we used a high throughput laser nephelometry automated microplate system to study the impact of abiotic factors on the growth of the so far described *Bisifusarium* species (n=7).

Fungal growth kinetics were determined in liquid medium (Potato Dextrose Broth) under different temperature (0 to 50°C), pH (2 to 14) and water activity (0.86 to 0.99) conditions. Both lag phases and growth rates were determined for each condition. This methodology is useful to determine cardinal values for growth of a given fungus and develop specific growth predictive models to better understand how a fungal species adapts to its environment.

From field to process: how storage selects toxigenic *Alternaria* spp. in apples

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Apples are a major crop in Argentina. The main destination of the fruits are export trade, internal commerce and industrialization. Apple fruits are susceptible to fungal contamination in the field as well as in the postharvest stage, with the consequent spoilage and severe economic loses. When mycotoxigenic fungi prevail as contaminants, an implicit health risk is associated. Fruits below quality standards for fresh consumption are transferred to by-products, mainly fruit concentrates, which are either exported or used in different local food industries. Before processing, fruits can be stored in refrigerated chambers for up to 9 months. *Alternaria* is the main causal agent of mouldy core (MC), a disease usually unnoticed in the visual inspection performed by apple concentrate industries for the detection of *P. expansum* blue rot. Given the wide variety of toxic metabolites produced by *Alternaria* spp., the incorporation of mouldy fruit to the process line is of concern regarding health risk.

The objective of this study was to characterize the apple fruit spoilage mycota and analyse its changes over storage, with special focus on mouldy core disease. A total of 240 apples from the Red Delicious variety, grown in Patagonia, Argentina, were collected and analysed; 140 were freshly harvested (H) and 100 had been stored (S) during 9 months in a refrigerated chamber (0-3ºC). For fungal isolation, a portion of damaged tissue from the fruit was transferred to Dichloran Cloramphenicol Malt Extract Agar (DCMA) plates. Fruits were disinfected, cut in half, and when mouldy core was detected, it was transferred to DCMA. After incubation at 25ºC for 7 days, fungal genera were identified.

From the 140 H apples, 120 (86%) showed external fungal lesions, and only 20 (14%) were undamaged. The incidence of MC was of 34.3% within this group. *Penicillium* spp. was the most frequent genus (54% infection), and it was mainly isolated from external lesions (43%). *Alternaria*, the second in frequency (41% infection), was isolated in similar proportions from external lesions (20%) and MC (21%). Other genera were *Geotrichum*, *Rizhopus*, *Mucor*, *Nigrospora* and *Trichoderma* (external lesions only), and *Cladosporium*, *Fusarium*, and *Epicoccum* (mostly external, low % of MC). Fungal contamination increased in stored apples (S); only 3/100 were undamaged, 48% had external lesions and 51% MC. *Alternaria* was predominant (60%), and mainly causing MC (46% infection). *Penicillium* took the second place, predominantly from external lesions (30%). *Cladosporium* and *Botrytis* were the only other genera found in these fruits.

Long-term cold storage selected *Alternaria* and *Penicillium* over non-toxigenic genera and inverted their frequency of contamination. MC caused by *Alternaria* spp. increased its incidence during storage. As MC fruits are more likely to be incorporated to the process line, these results suggest a high risk of contamination of apple by-products with *Alternaria* toxic metabolites.

The physiology of food-borne fungi and organic acid production in response to environmental conditions

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Food spoilage and food waste are of great concern for society. In order to prevent food spoilage by fungi it is important to build knowledge on the physiology of food-borne fungi. Therefore, the effect of water activity (aw), temperature and pH was studied on 26 food-borne fungi, including *Aspergillus niger, Mucor plumbeus, Penicillium roqueforti*, and *Paecilomyces variotii*. This was done by measuring growth in diameter and determining the relative growth speed for each environmental condition. Agar media with added fructose was prepared to adjust the aw in the range of 0.80-0.99 aw. The temperature range used was 0-40 °C. Growth on agar with adjusted pH (2-10.5) showed that fungi can adapt their environment by changing the pH. Therefore, the organic acid production of eight species was studied in more detail. Fungi were inoculated on malt extract broth with a pH of 8. The organic acid production (e.g. oxalic acid, gluconic acid, citric acid, and fumaric acid) by each species was determined daily for 7 days using HPLC. The pH was lowered only slightly, between 7 and 7.5 pH, for Aspergillus versicolor and *Mucor plumbeus*, while Aspergillus niger was able to lower the pH down to 1.78 in a non-buffered medium and down to 2.16 in tricine buffered medium. This shows that fungi are able to adapt their environment by excreting different types of acid, giving them a potential advantage over other microorganisms.

SESSION 4 CONTROL AND BIOCONTROL OF FOOD- AND AIRBORNE FUNGI, MYCOTOXIN PRODUCERS AND HEAT RESISTANT MOLDS

Biocontrol of Fusarium graminearum and deoxynivalenol accumulation during the micromalting process

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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*, cause *Fusarium* Head Blight in barley and produce quality and safety reduction during the malting process due to fungal growth and mycotoxin contamination. The aim of the present study was to evaluate the biocontrol ability of *B. velezensis* RC218 to reduce *F. graminearum ss* and DON accumulation during micromalting. The results showed that after the steeping stage there was an increase in the *F. graminearum ss* DNA content in both control treatments, positive and negative. Among the other stages, a decrease of the *F. graminearum* levels was observed, which could be related to the changes in the ecosystem that occur during the micromalting process. The application of the biocontrol agent during the steeping stage showed reduction in the *F. graminearum ss* levels at the end of this stage. However, a higher effectiveness of the biocontrol agent was observed at the end of the micromalting process, when *B. velezensis* was applied during the germination stage. Deoxynivalenol accumulation was reduced in both conditions, in the steeping and germination stage. At the end of the process it was observed that the quality parameters were not affected.

This activity has been supported by the EU Project MycoKey N. 678781 (http://www.mycokey.eu/)

Suppressing *Fusarium graminearum* and mycotoxins by application of microbial antagonists on infected crop residues

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The orientation towards sustainable agricultural systems requires innovative and integrated methods for the control of Fusarium Head Blight (FHB) in wheat to reduce the risk of mycotoxins that contaminate food and feed. In consequence, preventive actions against the dominating pathogen *Fusarium graminearum* using biological control agents (BCA) on infected crop residues could contribute to reduced applications of synthetic fungicides. Given the vast amount of candidate BCAs however, efforts need to focus on microbes with a proven activity against mycotoxin accumulation and a saprophytic lifestyle that is adapted to the environment. Within the scope of the Horizon 2020 project MycoKey, this project thoroughly investigated the ability of the fungal species *Clonostachys rosea* and *Trichoderma atrobrunneum* to suppress *F. graminearum* on maize residues and thus to reduce mycotoxins.

At first, a laboratory experiment was conducted to confirm the antagonistic activity of *C. rosea* strain 016 on maize stalk pieces infected with *F. graminearum*, either 48 hours before, simultaneously or 48 hours after the treatment. In contrast to other fungal candidates, *C. rosea* strain 016 completely inhibited the formation of perithecia as well as the discharge of ascospores. Subsequently, a field

experiment was carried out in 2016/17 and 2017/18 to compare the efficacy of formulations of *C. rosea* strain 016 and *T. atrobrunneum* strain Th908. The collected data included *Fusarium* spore dispersal during the infection period, disease symptoms, mycotoxin content, as well as the incidence of *Fusarium* species and *F. graminearum* DNA in harvested grains. The treatments with *C. rosea* strain 016 resulted in significantly lower FHB symptoms and reduced the deoxynivalenol content in harvested grains by up to 82% in the first and by up to 90% in the second year. Likewise, zearalenone was reduced by up to 80% in the first and by up to 90% in the second year. In conclusion, the results confirm the great potential of *C. rosea* to reduce FHB infections, which will be further investigated in on-farm experiments in the future.

Chasing Fusarium graminearum throughout its entire life cycle using botanicals: an in vitro approach

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Fusarium Head Blight (FHB) is one of the most important cereal diseases worldwide causing significant yield reductions and severe contaminations of harvested products with mycotoxins. Worldwide, *Fusarium graminearum* (FG) is one of the most common FHB causing species in wheat and barley cropping systems. By employing a unique approach, we assessed the control efficacy of different plant-based products (i.e. botanicals) on essential parts of the entire fungal life cycle using three FG strains (i.e. FG0410, FG2113 and FG1145). The botanicals included aqueous extracts from white mustard (*Sinapis alba*) seed flour (Pure Yellow Mustard (PYM) and Tillecur® (Ti)), as well as milled Chinese galls (CG). At 2% concentration (w v^{-1}), PYM and Ti completely inhibited the mycelium growth of all FG strains, while at 1%, CG reduced the growth by 65-83%, depending on the strain. Furthermore, PYM and Ti greatly inhibited the germination of both conidia and ascospores at 2% w v^{-1}, while CG was only effective against conidia germination. Perithecia formation of FG0410, but not of FG2113, was suppressed by all botanicals. Moreover, application of botanicals on mature perithecia led to two- to four-fold lower discharge of ascospores. Using liquid chromatography (LC) with diode array detection, we quantified the principal glucosinolate component sinalbin of PYM and Ti. LC time-of-flight mass spectrometry was used to demonstrate that the bioactive matrix of CG contains different gallotannins as well as gallic and tannic acids. Possible antifungal mechanisms of the botanical matrices will be discussed. The results of this study are promising and suggest that the examined botanicals should be further tested in crop protection programs against FHB, targeting different developmental stages of the fungus.

Zero Aflatoxin – Development and implementation of antifungal strategies to increase food safety in sub-Sahara Africa

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Africa is known as the world's second largest continent with a human population of about 1.2 billion people. Due to its geographical position, Africa has a relatively warm and humid climate. This climatic condition provides fungi such as *Aspergillus flavus* and *A. parasiticus* optimal growth support and can lead to a high load of food and feed with the strong carcinogenic mycotoxin aflatoxin. Since aflatoxin may be a frequent cause of death, especially for children, the elderly and the weakened, the development of sustainable strategies against fungal infestation is of utmost importance. Especially maize is a food that is very popular and commonly consumed in Sub-Saharan-Africa. Irrespective of the decades

of engagement of various initiatives to reduce fungal contamination, the problem still could not be solved. Furthermore, chemically based fungicides are too expensive for most small farmers and could form unwanted residues in the environment. Thus, only biological approaches will ensure a sustainable decrease in contamination of maize with the mycotoxin aflatoxin and thus an increase in food and feed safety. One such strategy is the application of the mycoparasitic fungus *Trichoderma harzianum* which itself is not able to produce harmful mycotoxins but actively attacks mycotoxin producing prey-fungi such as *A. flavus* and *A. parasiticus* which leads to disintegration and degradation of their filaments. This aspect additionally increases the nutritional value of the soil compartment. Another possible strategy is the spray application of an aqueous solution containing coumarin (benzopyrone chemical class). Plant parts containing coumarin concentrations up to 50 ppm are allowed as supplements in food. Coumarin inhibits structural similar benzopyrone-based mycotoxins such as the furano-coumarin, aflatoxin, on a mechanistic action comparable to negative feed-back response without affecting the growth of the fungus itself. These results and additional results gained in the actual research consortium project "AflaZ" together with German and Kenyan partners give hope that one day the aflatoxin contamination could substantially be reduced by researchers.

Fungal diversity and biocontrol of aflatoxins in GM- and non-GM maize cultivars

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GM-maize with either pest or herbicide resistance or both are now commonly grown in many countries, especially in the USA and South America. There is however, very little information on the fungal diversity and potential for control of *Aspergillus flavus* and aflatoxins in such cultivars when compared with their isogenic non-GM cultivars. This study has examined 6 different non-GM and related GM-maize cultivars to quantify the diversity and dominance of different fungi with a focus on toxigenic species. The secondary metabolite profiles in these related groups of cultivars were also compared. Some of the fungal isolates including non-toxigenic *A.flavus* isolates were screened for potential control of aflatoxin production by toxigenic strains *in vitro* and *in situ*. These studies showed that some atoxigenic strains of *A.flavus* regardless of inoculum ratios were able to inhibit aflatoxin production and maize contamination under different water availability x temperature conditions. Subsequent studies examined the relationship between biocontrol and simulated pest damage in related pest resistance GM and the equivalent non-GM cultivars. In addition, potential impacts on resilience of biocontrol under climate change conditions was examined.

Controlling the spoilage of fruit purees by heat-resistant moulds (HRMs): Diversity of HRMs during processing and inhibition of their growth during storage

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Whilst pasteurized high-acid fruit products, such as fruit purees, are generally considered to be microbiologically stable, heat-resistant moulds (HRMs) have been reported to cause spoilage. While several studies have been made to develop appropriate strategies to extend the shelf-life of high-acid fruit products by i.e. inactivating and/or preventing the germination and growth of ascospores of HRMs, the HRMs remain a challenge for fruit producers worldwide. This study was conducted to (i) determine the occurrence and diversity of HRMs throughout fruit purees processing; (ii) investigate the effect of processing on the levels of HRMs and (iii) determine conditions inhibitory for the growth

of HRMs. A total of 242 samples of raw, intermediate and finished fruit products were collected from two processing plants located in Belgium (strawberry puree) and the Netherlands (apple puree). Ascospore forming moulds were detected in 90.9 and 48.7% of strawberry and apple puree samples, respectively. Twelve species were identified by molecular based methods which belonged to the genera Byssochlamys, Aspergillus-type ascospores, Talaromyces and Rasamsonia. Aspergilli with Neosartorya-type ascoma were predominant and present throughout the samples collected from both processing plants. Thereafter the growth limiting conditions regarding °Brix/Aw, temperature and oxygen (O^2) level were determined for six HRMs isolated from the samples collected at the two processing plants. With these data in hand, the combined effects of O_2 (0.1-1.0%), pasteurization intensity (95-105°C/15 sec.), A^w (0.87-0.89), storage temperature (10, 22, and 30°C) and time to visible growth (0-91 days) on the growth of N. fischeri was then determined. Growth was not observed at 10°C at all combinations of conditions assessed. Inhibition of the outgrowth of N. fischeri as a consequence of the depletion of O_2 during incubation was more notable when the other factors were sub-optimal, such as at reduced Aw's. As expected, Aw plays a major role in the inhibition of the germination of ascosopores and subsequent mycelial outgrowth.

Potential of antifungal lactic acid bacteria combinations as bioprotective cultures in pilot scale dairy products

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Food spoilage is a major issue for the food industry, leading to important food waste and economic losses. Dairy products, despite their microbial stability, are susceptible to acid tolerant fungal contaminants. In this context, antifungal cultures are of growing interest as an alternative to chemical preservatives or a complement tool to hurdle technologies. To develop antifungal cultures for dairy product biopreservation, firstly, the antifungal activity of 32 lactic acid bacteria and propionibacteria strains was screened alone, and then in combinations (for 5 selected lactobacilli strains). This screening step, performed *in vitro* in yogurt and cheese models against four major spoilage fungi previously isolated from contaminated dairy products (*Penicillium commune*, *Mucor racemosus*, *Galactomyces geotrichum*, and *Yarrowia lipolytica*), allowed the selection of two binary combinations of interest (A1 and A3). These selected combinations were then tested *in situ* as adjunct cultures in sour cream and semi-hard cheeses produced at a pilot scale to evaluate i) their antifungal activity during both challenge tests and shelf life tests, and ii) their impact on product organoleptic properties. In the tested conditions, the A1 combination delayed the growth of *P. commune*, *M. racemosus* and *R. mucilaginosa* for 2–24 days on sour cream depending on the antifungal culture inoculum, without effect on organoleptic properties at low inoculum level (10^6 CFU/mL). Moreover, the A1 and A3 combinations also delayed the growth of *P. commune* in semi-hard cheese for 1–6 days and 1 day, respectively. Antifungal cultures neither impacted the growth of starter cultures in both sour cream and cheese nor the product pH, although post-acidification was observed in sour cream supplemented with these combinations at the highest concentration (2.10^7 CFU/mL). The combination of both *in vitro* and *in situ* screening assays allowed developing 2 antifungal combinations exhibiting significant antifungal activity and providing future prospects for use as bioprotective cultures in dairy products.

Design and up-scaling of filters coated with zinc oxide nanoparticles in pear warehouses

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Fresh fruits can bring large economic benefits when they undergo a proper postharvest life. Storage of fruits for several weeks or months before marketing may cause disease problems if good postharvest handling procedures are not accomplished. Debilitating or contaminated environments should be avoided and lifeλshortening injuries prevented or at least minimised. The most common way to reduce the abundance of fungal spores in storage environments is by air filtration. In this study, the anti-fungal efficiency of filters (melt-blown and needle-punched) coated with ZnO nanoparticle solutions was assessed at a lab scale. The most effective coating conditions, i.e. nanoparticle concentration and coating time, for the inhibition of fungal conidial suspensions, *P. expansum* and *R. stolonifer*, previously isolated from pome fruits, were identified. Hereafter, a ventilation system in a pear storage warehouse was designed in order to place the coated air filters. Initial computational fluid dynamics (CFD) simulations were performed on an *in-silico* warehouse model having dimensions of a typical warehouse. The filters were designed in such a way to abate by the pre-defined pressure drop parameters. The filter prototype (needle-punched HS-Alpha Pak) coated by ZnO nanoparticles was produced and installed at the warehouse facilities. The antimicrobial efficiency of the developed prototype was assessed following an operation of six months. Assessments included: (1) passing sterile air through the filter and collecting any detached spores from the filter which resulted in limited fungal growth, (2) flooding the surface of each section with sterile Tween 80 suspension and scraped off with a sterile bent rod in order to detach spores eventually present onto the surface where no growth was observed in any of the filter‘s sections, (3) placing the filter on nutrient agar to assess surface contamination of the filter where all the flaks showed bacterial and fungal growth onto the medium‘s surface, albeit to a low level. These results indicate that the surface of the filters that is exposed to the air from the warehouse had some environmental contaminants. Their levels are quite low indicating that even on the surface the antimicrobial efficacy of the nanoparticles was pronounced.

SESSION 5: MYCOTOXINOGENIC FUNGI AND MYCOTOXINS

Mycotoxins in animal feed

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Animal feed is often an overlooked factor when it comes to animal health and welfare. The feed quality varies a lot depending on whether the animals are pets or production animals. Farmed mink only live 8-9 months before culling and feed quality is only challenged if the mink get gastrointestinal disorders and show stunted growth. Danish mink feed constitutes of offal from the fishing and meat industry mixed with corn gluten meal, soybean oil and extruded cereals. Our results show that mixed mink feed can contain cocktails of mycotoxins (fumonisins, deoxynivalenol, zearalenone enniatins and sterigmatocystin) and bacteria (*E. coli*, *Staphylococcus* and *Clostridium* spp.), which may not in themselves cause acute diseases in the animals, but may explain syndromes like wasting mink disease.

Comparative mycology and chemistry of apple and citrus fungi

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Apples in general have specifically associated fungi and bacteria that will infect them, including *Alternaria tenuissima*, *A. arborescens*, *Fusarium avenaceum* in the apple core and *Monilinia fructicola, M. fructigena*, *M. laxa*, *Penicillium expansum*, *P. solitum*, *P. crustosum* on the surface in addition to the bacterium *Alicyclobacillus acidoterrestris* in the apple. We wanted to examine whether these fungi were also present as endophytes in the less acidic apple leaves and twigs, and whether *Alicyclobacillus* was present as an endophyte. After surface disinfection a series of filamentous fungi and *Bacillus amyloliquefaciens* were rather consistently isolated. The fungi isolated were not the serious apple rot fungi such as *P. expansum*, *P. solitum* and *Monilinia* spp., but rather fungi such as *Alternaria tenuissima*, *Broomella acuta*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Fusarium torulosum*, and *Phoma* spp. These fungi produced secondary metabolites such as antibiotic Y, alternariol, tenuazonic acid, orevactaene, chaetoglobosin A, and chlamydosporol, but none of the fungi were able to inhibit *Bacillus amyloliquefaciens*. On the other hand *Bacillus amyloliquefaciens* strongly inhibited all the endophytic fungi isolates without exception. Chemical analysis (HPLC-DAD-MS) of the inhibition zones, using interference competition experiments showed that the *Bacillus* sp. produced antifungal (and antibacterial) lipopeptides such as iturins and fengycins. Furthermore some fungi such as *Cladosporium cladosporioides* produced extrolites that enhanced the growth of the Bacilli. We speculate that the apple tree recruit endophytic *Bacillus* spp. to keep the endophytic fungi in check. On citrus fruits we isolated *Penicillium italicum, P. ulaiense* and *P. digitatum* and some *Alternaria* spp in the seed core. The citrus leaves also contained *Bacillus* spp. and fungal endophytes. There was a big difference between the microbiology and chemistry of the three Penicillia from apples and the other three from citrus fruits.

A "snapshot" of biodiversity of Aspergillus section Flavi in Brazilian foodstuffs

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Aspergillus section *Flavi* stands out as of great concern for agriculture and the food industry since most of the species of this group are aflatoxigenic and have great adaptability to different conditions, which allows them to colonize innumerable habitats. The aim of the present work was to compare the distribution of biodiversity of *Aspergillus* section *Flavi* in three Brazilian foodstuffs: brazil nuts, rice and sugarcane isolated along the processing chain of each of these foods. Each sample was surface disinfected with 0.4% chlorine solution and plated directly onto Dichloran 18% Glycerol agar and incubated at 25 ºC for 5 days. For powder and liquid samples the dilution plate technique was used and plated on DG18 or DRBC with the same incubation conditions. After incubation, all fungal species belonging to *Aspergillus* section *Flavi* were isolated for further morphological and molecular identification. In brazil nuts, six aflatoxigenic species were found: *A. flavus, A. nomius, A. luteovirescens* (formerly *A. bombycis*), *A. arachidicola, A. pseudonomius* and *A. pseudotamarii*. The most common species was *A. nomius,* with all isolates capable to produce aflatoxins B_1, B_2, G_1 and G_2. *A. flavus* was almost equally common although only half of the strains produced aflatoxins under laboratory conditions, and exclusively aflatoxins B_1 and B_2. The other species were recovered at low numbers. The total aflatoxin levels found in samples varied from 0.1 µg/kg to 151 µg/kg at different stages of the brazil nut production chain (rainforests, processing plants, street markets and supermarkets). In rice, five species were found belonging to *Aspergillus* section *Flavi: A. flavus, A. caelatus, A. novoparasiticus, A. arachidicola* and *A. pseudocaelatus*. This was the first report of occurrence of these last three species in rice and rice plantation soil. Only 17% of *A. flavus* isolates from rice were able to produce aflatoxins B_1 and B_2, but 95% produced kojic acid and 69% cyclopiazonic acid. Less than 14% of the rice samples were contaminated with aflatoxins. Among the isolates of *Aspergillus* section *Flavi* in sugarcane as well as in by-products (molasses, sugar, yeast cream and dried yeast), *A. novoparasiticus* and *A. arachidicola* were predominantly found. This was the first report of contamination of sugarcane by these two species. Most samples, with the exception of sugar, showed some aflatoxin contamination. The highest level was in dried yeast. In conclusion, great diversity of *Aspergillus* section *Flavi* species was found. Most of the species were potentially aflatoxigenic; however, the levels of contamination found in the samples were generally low. An intriguing fact was the strong association of some species with their substrates of origin: *A. novoparasiticus* to sugarcane; *A. nomius* to brazil nuts and *A. flavus* to rice. It can be observed that these species are present from the crop, acting in a mutualistic way. The acquisition of mutualistic symbiosis may provide new ecological opportunities for evolutionary diversification, which may lead to co-adaptation; however, this is only a conjecture that requires in-depth studies.

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Fumonisin occurrence in wheat and wheat by products

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Wheat is the most important cereal consumed by the Argentine population. It can be milled to produce flour and semolina mostly used for bread, pasta or other bakery products elaboration. It is remarkable that wheat flour consumption per capita in Argentina was estimated at 86 kg/habitant/year in 2016. The main pathogen associated with *Fusarium* Head Blight (FHB) wheat disease in common and durum wheat in Argentina is *Fusarium graminearum*, also deoxynivalenol (DON) has been reported in both

types of wheat. However, when *F. graminearum* was not the main species isolated from wheat, the predominant *Fusarium* sp. was *F. proliferatum*, and durum wheat grains were contaminated with fumonisins (FBs). Fumonisins are toxic fungal metabolites that have been epidemiologically associated with oesophageal cancer and neural tube defects in some human populations. A survey was carried out to determine FBs contamination in 135 common and 40 durum wheat samples in the main wheat production area of Argentina using HPLC-MS/MS. Also, FBs occurrence was analyzed in 46 wheat-based products obtained from local supermarkets. Considering that *F. proliferatum* could be responsible for fumonisin presence in wheat and wheat-based products, two ecophysiological surveys were made using srains isolated from wheat, grown in a wheat based-medium and in irradiated wheat grains. Moreover, relative *FUM8* and *FUM19* genes expression was analyzed under different a_w and temperature using a wheat-based medium. As a result, 93% of total wheat grain samples showed FBs contamination, with levels ranging from 0.15 to 1304.39 ng/g, being FB_1 the fumonisin most frequently found. Most samples were contaminated with FB_1 and FB_2. Four and 9 common and durum wheat samples, respectively, showed no contamination with fumonisins, while 9% and 7.5% of common and durum wheat samples, respectively, showed levels higher than 1000 ng/g. All wheat-based products were contaminated with FBs (levels ranging from 0.04 to 18.94 ng/g, median: 1.43 ng/g). A large number of samples had higher levels of FB_2 than FB_1. Optimal a_w levels for *F. proliferatum* strains growth rate ranged from 0.995 to 0.96 at 25–30 °C, when a_w was lower than 0.90, strains were not able to grown. The maximum amounts of FBs were obtained at higher levels of a_w (0.995, 0.99 and 0.98) at 25 or 15 °C depending on the substrate and the strain. FB production profiles for each particular strain were related to incubation temperatures. *FUM* genes expression at 25 °C correlated with FBs production, regardless the a_w, while at 15 °C FBs production was lower than at 25 °C, but *FUM* genes were expressed as at 25 °C. This is the first report on natural FBs presence in common wheat grains and wheat-based products in Argentina. Also, the present study provides very useful guidelines for facilitating effective management of predicting risk for growth and mycotoxin production during ripening, harvesting and storage of wheat.

Transfer of deoxynivalenol and deoxynivalenol-3-glucoside from barley to boiled wort

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Fusarium Head Blight disease (FHB), which is highly occurring on barley crops, represents an important issue for farmers and brewers. Firstly, because it affects grain quality and production yield (*e.g.* germination potential) and secondly, because it is usually accompanied by mycotoxin accumulation, which are proven toxic for humans and animals. The fate of the most occurring trichothecene, deoxynivalenol (DON), and its modified form, deoxynivalenol-3-glucoside (DON-3-Glc), was studied through malting, mashing and wort boiling steps. Barley and malt contaminated with DON and DON-3-Glc produced by a toxigenic strain of *Fusarium graminearum* were used for the study. Malting was globally characterized by an increase in both DON and DON-3-Glc compared to their level before the process, although different tendencies were registered: almost 75% of DON was washed-out during steeping, an increase with a subsequent decrease during germination were observed having a peak level after 48h of the process for both toxins, followed by another increase during kilning by almost 20 and 107 % for DON and DON-3-Glc, respectively. A release of both DON and Don-3-Glc from malt matrix and their transfer to the wort was reported during mashing. Interestingly, a significant reduction in the spent grains was observed after 15 min at 45ºC (almost 100% decrease of DON and DON-3-G in the spent grains with their simultaneous increase in the sweet wort), the next stages not leading to a significant change in mycotoxin concentration. Both DON and DON-3-Glc were reduced to their initial level contained in malt before mashing or even lower, however in none of the sample this reduction was complete.

Production and migration of ochratoxin A and citrinin in *Penicillium verrucosum* molded Comté cheese

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Penicillium verrucosum is a fungal species contaminating foods, such as cheeses, that can lead to significant economic losses and food waste. This species can produce toxic extrolites, including ochratoxin A (OTA) and citrinin (CIT), which can cause serious physiological effects in humans. The objective of this study was to develop an experimental plan to evaluate the migration of these two mycotoxins in *P. verrucosum* artificially contaminated Comté cheese.

Multiple strains belonging to known mycotoxin-producing species that can contaminate cheeses were first compared for growth and mycotoxin production on cheese. A strong OTA/CIT-producing P. verrucosum strain was then selected and a spore suspension was inoculated onto Comté cheese cubes and incubated at 8°C (for 42 days) or 20°C (for 28 days) to mimic typical storage at the consumer level. Fungal growth was monitored and sampling for OTA/CIT determination was carried out at regular time intervals. Mycotoxins were then extracted and quantified using LC-QTOF from 2 mm thick cheese slices cut out from 4 cm3 cheese cubes as follows: 0-2, 2-4, 4-6 and 6-8 mm.

Using this methodology, CIT and OTA production and migration were monitored in Comté cheese for both storage temperatures. A clear shift in secondary metabolite biosynthesis was observed for samples stored at 8°C as CIT production was highest at 14d, then decreased, while OTA production started at 28d. For samples stored at 20°C, simultaneous production and migration of both metabolites was observed from 7d on.

The obtained data can be used for risk assessment and recommendations for consumers in the case of mold contaminated cheeses have been proposed.

Quality control of mycotoxin producing fungi contamination in cocoa beans and its product: Case study in Indonesia

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As an agrarian country, Indonesia is the third highest producing country of cocoa beans. With the high productivity, cocoa beans are not only sold in the form of fermented bean but also in processed form such as chocolate bar and confectionary product. Currently, cocoa bean products can be supplemented with probiotics to meet the high demand of health benefit food. However, cocoa bean and its product from Indonesia has low quality due to poor handling from farm to table. The main cause of low quality of Indonesian cocoa bean is mycotoxin producing fungi contamination that can come either during fermentation or drying process of cocoa bean. A potential black aspergilli ochratoxin producer has been successfully isolated from cocoa beans in Yogyakarta, Indonesia and identified as *Aspergillus carbonarius*. Analysis showed that cocoa beans were contaminated with ochratoxin A as much as 57.68 ppb. Therefore, in this paper review we elucidate the strategies of quality control of cocoa bean from fermentation until the final product as an action to cope with the low quality of cocoa bean and its products from Indonesia. Particularly, by using *Lactobacillus plantarum* as a starter culture to inhibit fungal growth.

SESSION 6: SANITATION OF FOOD SPOILAGE FUNGI

Adequate sanitizers for controlling food spoilage fungi

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The deterioration of food by fungi is a major problem for the food industry, generating considerable economic losses. In this context, the presence of fungi in dairy and bakery factories is responsible for losses of up to 5% of their manufactured products, and also produces losses in cured meat products. *Penicillium* is one of the main fungal genera responsible for the spoilage of products from these industries. It is well established that the airborne fungal load in facilities plays a crucial role in the contamination of food products and prompt the occurrence of early deterioration. An effective way to avoid this problem is through the use of chemical sanitizers to control environmental contamination in dairy, baking and cured meat products industries, preventing contamination of the product during its elaboration. However, information regarding the sensitivity of deteriorating fungi in these products is limited. Thus, this study aimed to evaluate the antifungal activity of different classes of chemical sanitizers with permitted use in the food industry against the main fungi involved in the deterioration of the products mentioned above. The tests were carried out according to the protocol for tests of the antifungal effect of chemical sanitizers of the European Committee for Standardization (CEN), with adaptations. Different strains of 4 fungal species isolated from spoiled bakery products*: P. roqueforti, P. paneum, H. burtonii* and *A. pseudoglaucus*; 2 species isolated from cheeses: *P. roqueforti* and *P. commune*; and 3 isolates from deteriorated cured meat products: *A. westerdijkiae*, *P. polonicum* and *A. pseudoglaucus*, were tested against five sanitizers at three concentrations of use: peracetic acid (0.15%, 1.5%, 3%); biguanide (2%, 3.5%, 5%); benzalkonium chloride (0.3%, 2.5%, 5%); sodium hypochlorite (0.1%, 0.5%, 1.5%); quaternary ammonium (0.3%, 2.5%, 5%). In general, the fungi showed a varied susceptibility to the tested sanitizers, with the exception of *A. pseudoglaucus* strains, that showed a higher resistance when compared to the other fungi tested. Sodium hypochlorite and peracetic acid were the most effective sanitizers considering the genera, species and concentrations evaluated in this study. On the other hand, biguanide should not be used when the aim of a sanitizing program is the fungal control.

Sanitization by hydrogen peroxide and peracetic acid: are heat resistant moulds the best choice for bio-validations of food packaging and machineries?

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Within a food aseptic processing, packaging and machineries sanitization is aimed at the inactivation of the microbial population. It can be performed using heat, chemical sanitizers or physical methods. Among these, sanitization by hydrogen peroxide or peracetic acid is the most widespread practice. At an industrial level, when a sanitization process of packaging/machines for pasteurized products (pH > 4.5) distributed in the cold chain or acid products (pH ≤ 4.5) distributed outside the cold chain must be assessed, the effectiveness of these substances is usually tested by means of selected microorganisms: *Aspergillus niger* ATCC 6275, *Aspergillus brasiliensis* ATCC 16404, or *Bacillus atrophaeus* SA 22, according to VDMA guidelines. This choice has been probably made because they are so far considered the microorganisms most resistant to these chemicals. Nevertheless, recent findings about HRM occurrence on both packaging materials and industrial environments have lead us to assess their resistance to chemical sanitizers, presuming a greater resistance to chemical stresses of fungal genera such as *Talaromyces* or *Aspergillus* (with eurotium or neosartorya-type ascomata) and *Chaetomium*, compared to test microorganisms used by food industries for their validations. For both sanitizers,

tests were carried out with or without a supporting material (alluminium, tin plate, PET, or HDPE).

With peracetic acid (1000 ppm), at 40°C the most resistant microorganism proved to be *Chaetomium globosum*, whose decimal reduction time (70 minutes) was markedly higher than the ones calculated for other microorganisms.

With hydrogen peroxide (40%), the most resistant microorganism proved to be *Talaromyces bacillisporus*, whose decimal reduction time (e.g. D_{50}=23 s; D_{55}=10s; D_{60}=5swithout a supporting material) was higher than the ones calculated for other microorganisms, except for *B. atrophaeus* that proved to have lower D_T up to 60-65°C, but a higher z value. With both sanitizers, tests on different supporting materials showed different results, probably due to varying adhesion forces involved, even though no changes in chemical resistance rank of microorganisms tested were observed.

For practical purposes, spore-forming moulds could be therefore considered more suitable as test microorganisms than those actually suggested, their use leading to more realistic results during validations of sanitization processes on packaging/machines for refrigerated products (pH > 4.5) or non-refrigerated acid products (pH $\leq$ 4.5).

Mould spoilage of heat-processed/hot-filled beverages: When everything goes wrong!

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Nowadays, consumers desire less processed, non-preserved products. Controlling their spoilage can be challenging. One way to control mould spoilage in these types of beverages is by pasteurization. However, even pasteurization can be ineffective in preventing spoilage of hot-filled pasteurized beverages by both heat-resistant and heat-sensitive moulds. Heat-resistant moulds (HRM) have spores that can survive both the pasteurization treatment and hot filling. These spores (ascospores) can be found in the ingredients, packaging and the processing environment. The contamination of these products by non-heat resistant moulds occurs after the filler due to faulty caps, seal, too much elapsed time between the filler, at the cooling tunnel, or excessive moisture on the bottle threads. In either case, vacuum does not form or is delayed, allowing cooling tunnel water to enter inside of the bottle. Biofilm on the cooling tunnel surfaces as well as the air in the filler/capper room can be sources of these microorganisms. Excessive head space in the bottle can cause moulds inside the lid to grow on the lid or on top of the liquid. Thread mould can be formed if there is residual liquid between the thread of the bottle and the cap. Biofilm build-up in the cooling tunnel must be avoided since many bacteria, yeasts and even some moulds thrive in wet conditions. However, biofilm can be very difficult to remove using conventional cleaning methods. Sometimes, even when the beverage manufacturer thinks that all the preventive measures are in place, many things can go wrong, and different types of spoilage can take place at the same time. This presentation will review a case study where everything went wrong.

SESSION 7: FUNGAL GENETICS AND GENOMICS

Changes in the expression of a gene involved in cell wall integrity are related to mycotoxin production in filamentous fungi in food

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Spoilage fungi and mycotoxigenic fungi are able to colonise a wide range of ecological niches including soil, plants and foods. External conditions and antifungal compounds may trigger different signal cascades within the fungal cell that culminate in changes at the transcriptional level, leading to the production of compatible solutes that enable the enzyme systems to function under the environmental conditions. It has been suggested that these compounds may act as stressors and provoke an increase of mycotoxin production. Fungi have different intracellular pathways that help them coping with challenging external conditions. Among them, the cell wall integrity pathway (CWI) is activated in response to cell wall stresses due to different food-related environments. So far, little is known about the relationship between the activation of the CWI pathway and mycotoxin production by important economic filamentous fungi commonly found in various cereals and fruits. The objectives of this work were to: a) evaluate the effect of external conditions (temperature, water activity) and antifungal compounds (fungicides, biocontrol agents) on growth, mycotoxin production and changes in the expression of the *Rho1* gene, one of the main regulators of CWI pathway by some *Alternaria* species and *Aspergillus ochraceus* in model systems based on wheat, tomato and raisins; b) analyse the relationship between *Rho1* gene expression and both the growth and mycotoxin production by the target filamentous fungi. Results showed that temperatures, water activity and the nature of the fungicide have different influence on both growth and mycotoxin (ochratoxin A, alternariol, alternariol monomethyl ether and tenuazonic acid) production. In addition, it has been shown that changes in the CWI pathway and the accumulation of ochratoxin A and *Alternaria* spp. mycotoxins seem to be related under specific environmental conditions. These findings will be useful in developing new strategies to efficiently control toxigenic fungus spoilage in cereals and fruits.

Regulation of mycotoxin biosynthesis by *Penicillium expansum* as an important determinant of the colonization capacity

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Penicillium expansum is an important post-harvest pathogen of fruits, especially apples. It is able to produce the two important mycotoxins patulin and citrinin. For both mycotoxins it was shown that their biosynthesis supports the colonization of the natural habitat. Mutants in biosynthetic genes of both toxins (*pks*CT, *pat*K) have a reduced capacity to colonize apples. Patulin is usually produced very early during growth and at much higher amounts compared to citrinin. Citrinin in contrast, can analytically be detected only lately during the infection process, generally in correlation to the production of aerial mycelium. The biosynthesis of mycotoxins is strongly dependent on the substrate and various environmental parameters. The most important parameters are temperature, water activity and pH. Changes in the pH of the substrate are sensed by the PACC signal cascade which transfers the signal to the transcriptional level thereby regulating certain responsive genes which also might include

mycotoxin biosynthetic genes. Fine-tuned regulation of mycotoxin biosynthesis under conditions of the natural habitat is important for the fungus for the colonization of the substrate. For *Penicillium expansum* it could be shown that the PACC regulated citrinin biosynthesis supports adaptation and colonization. The *pac*C gene was inactivated and it was demonstrated that the resulting *P. expansum* transformants had a reduced colonization capacity on apples. For *P. expansum* it was shown that there is an interplay between PACC regulation and the transcriptional activator of the citrinin biosynthesis gene cluster, CTNR. A higher expression of the *ctn*R gene could override PACC regulation which results in constitutive biosynthesis of citrinin. Interestingly, according to literature data, also the biosynthesis of patulin is regulated by PACC.

Genomic diversity in ochratoxigenic and non ochratoxigenic strains of *Aspergillus carbonarius*

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Ochratoxina A (OTA) is a mycotoxin with nephrotoxic effects that has been associated to kidney problems in both livestock and human populations and it can be found in a variety of common foods and beverages. This mycotoxin is produced by several species of *Penicillium* and *Aspergillus* among which *Aspergillus carbonarius* is recognized as the main OTA source on grapes and derived products. OTA production is a very consistent property of *A. carbonarius* and nearly 100% of the isolates of this species produce OTA. Little is known about the genes involved in the OTA biosynthetic pathway although recently a consensus OTA biosynthetic pathway has been proposed in *Aspergillus ochraceus*. In *A. carbonarius*, a hypothetical OTA gene cluster has been characterized. This cluster contains three genes, a nonribosomal peptide synthetase (*AcOTAnrps*) gene, a polyketide synthase (*AcOTApks*) gene and a halogenase gene (*AcOTAhal*), directly related to OTA biosynthesis. There are two other genes located in the same genomic region that could play a role in the biosynthesis pathway as part of the OTA cluster. These genes were a cytochrome p450 monooxygenase (*AcOTAp450*), and a transcription factor (*AcOTAbZIP)*. However, the OTA cluster remains not completely defined and most of the regulatory aspects underlying OTA production remain unclear. In the present study, we present the genome resequencing of four *A. carbonarius* strains, one OTA producer and three atypical non-OTA producing strains. These strains were sequenced using Illumina technology and compared with the genome reference Acv3. Besides this main objective, and due to the fact that three of these strains do not produce OTA, we performed some new specific bioinformatics analyses in genes involved in OTA biosynthesis. We focused these analyses on nonsense and missense mutation detection, and also in to identify whether large DNA sections of the reference genome Acv3 or of the newly sequenced OTA producing strain were absent in the genome of the three non-OTA producing strains. Regarding the genes potentially involved in OTA biosynthesis, the OTA-producer strain showed variants in *AcOTApks*, *AcOTAnrps*, *AcOTAbZIP* and *AcOTAp450*. In atoxigenic strains only five common missense variants in *AcOTApks* gene were found. Although some gaps of more than 1,000 bp were identified in non-ochratoxigenic strains, no large deletions in functional genes related with OTA production were found. Moreover, the expression of five genes of the putative OTA biosynthetic cluster was down regulated under OTA-inducing conditions in the non-ochratoxigenic strains. Knowledge of the regulatory mechanisms involved in OTA biosynthesis will provide a deeper understanding of these nonochratoxigenic strains.

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PCR-based distinction of high and low toxic strains in *Stachybotrys chartarum*

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Stachybotrys chartarum is frequently isolated from damp building materials or improperly stored animal forage as well as dried culinary herbs. Human and animal exposure to this mould has been linked to severe health effects such as the sick-building syndrome and stachybotryotoxicosis, respectively. Two distinct chemotypes of the fungus were described that produce either highly cytotoxic macrocyclic trichothecenes, e.g. satratoxins (S-type), or the less toxic atranones (A-type). To further examine these characteristics, PCR primers were developed to detect all genes in the gene clusters coding for satratoxins or atranone in the respective chemotypes. Presence/absence of each of the 21 *SAT* (satratoxin) genes and 14 *ATR* (atranone) genes was examined in a total of 27 strains of chemotype S (n=19) and chemotype A (n=8) and in one strain for *S. chlorohalonata*. Analyses revealed that several non-satratoxin producing strains contained marker genes for both atranone and satratoxin biosynthesis. Further, the results suggest that truncations occur at different locations within the *SAT* gene cluster in non-satratoxin producing S-type strains. Obviously, the observed truncations render satratoxin production impossible in the respective isolates. Based on the detected genetic differences we propose a new genotype-based concept for differentiation of *S. chartarum* isolates using a triplex PCR (tPCR) that defines three genotypes: genotype A (atranone genotype, producing no macrocyclic trichothecenes, having no *SAT* genes), genotype H (hybrid genotype, producing no macrocyclic trichothecenes, having a truncated cluster of *SAT* genes), and genotype S (satratoxin genotype, producing macrocyclic trichothecenes, having the complete cluster of *SAT* genes and no *ATR* genes).

ABSTRACT POSTERS

01. Effect of Sodium meta-bisulphite (NaMBS) on *in situ* populations and aflatoxin B_1 production by *Aspergillus flavus* in chillies

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Inefficient drying of chillies can result in aflatoxin (AFs) contamination. This may be exacerbated in store if the environmental conditions are not effectively controlled. The objective of this study was to examine the efficacy of different concentrations of sodium metabisulphite (NaMBS) for potential control of mycota populations and aflatoxin B_1 (AFB_1) contamination of whole chillies when inoculated with a toxigenic strain of *A. flavus* isolated from chillies. Studies included the addition of between 0-2000 ppm NaMBS or the use of commercial sheets impregnated with different concentrations (40, 70%) of the preservative (Tessara Ltd, Cape Town, S. Africa). The latter product involved the release of SO_2 over 48-72 hrs and then a slow release over the rest of the storage period. The efficacy of these treatments was examined at 4 different water activity levels (0.70, 0.80, 0.90 & 0.95) in stored whole chillies and chilli powder at 30 °C over a 20-day storage period. The total mycota populations and those of *A.flavus* were significantly reduced by using NaMBS in the chilli powder treatments. In addition the AFB_1 concentration was reduced. Use of the commercial sheets to provide a release of SO_2 into the storage containers with different levels of exposure (40 or 70%) also resulted in effective decrease in total mycota and *A.flavus* populations as well as AFB_1 contamination when compared to the untreated control, regardless of a_w level. The results are discussed in the context of intervention strategies to minimise AFB_1 in spices.

02. Solute and matric potential stress and *Penicillium verrucosum*: impacts on growth, gene expression and ochratoxin A production

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The objectives of this study were to examine the effect of ionic and non-ionic solute water potentials and matric potential on (a) in vitro growth, (b) expression of the toxin biosynthetic genes *otapksPV* and *otanrpsPV*, and (c) phenotypic ochratoxin A production by *P. verrucosum*. The optimum conditions for growth and OTA production were at -7.0 MPa (=0.95 aw) and -1.4 MPa (=0.99 aw) respectively, regardless of whether solute or matric stress was imposed. *P. verrucosum* was more sensitive to ionic solute stress with no growth at -21 MPa (=0.86 aw) while with non-ionic solute and matric stress growth still occurred. For OTA production the optimum was at -1.4 MPa (=0.99 aw) for both solute and matric potential. The amount of toxin produced decreased significantly as water stress was imposed. Very limited OTA production was observed at -14.0 and -21.0 MPa (= 0.90 and 0.86 aw) under solute and matric stress respectively. Relative gene expression of key toxin biosynthetic genes was quantified using reverse transcription quantitative PCR (RT-qPCR). The *otapksPV* gene was expressed over a wide range of ionic and non-ionic solute stress conditions from -1.4 to -14.0 MPa (=0.99 - 0.90 aw). The highest expression was with the non-ionic water stress treatments at -7.0 MPa (=0.95aw). However, the *otanrpsPV* gene was up regulated under matric stress, especially when the water was freely available (-1.4 MPa = 0.99 aw). This study has demonstrated that the growth behaviour of *P. verrucosum* in response to different solutes is quite different from that for OTA production. This species often contaminates cereals and survives on crop residue and in soil. This xerotolerant species is thus able to tolerate matric stress and as well as non-ionic solute stress and colonise crop debris to survive and

infect the subsequent crop. In terms of biosynthetic genes involved in OTA production there were some differences between solute and matric stress suggesting that this needs to be taken into account in determining the risk of OTA contamination from soi

03. Is it safe to scrape the mould off your food and eat what's underneath it? The example of growth and mycotoxin production of *Penicillium expansum* on apple jam and *P. verrucosum* on crème fraiche at 4, 8 and 15°C

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'Is it safe to scrape the mould off your food and eat what's underneath it?' could be a question for many consumers in private homes when their refrigerated food became mouldy. To answer this question, we initiated a study and followed the growth of *Penicillium expansum* on the surface of apple jam and *P. verrucosum* on light crème fraiche at 4, 8 and 15°C over time (up to 28 days). Production and distribution of fungal metabolites throughout the sample were analysed. The food sample was divided into 3 subsamples: A was 0-2 cm from the surface including the fungal colony, subsample B was 2-4 cm and subsample C was the rest from >4 cm from the surface. The growth of *P. expansum* was observed on the surface of subsample A in apple jam at all storage temperatures. Growth rates and lag times of *P. expansum* in apple jam were estimated at 4, 8 and 15°C. At 15°C after 14 days, patulin had migrated into layer B, where the concentration of 2490±680 µg/kg was two-fold greater than the tolerable daily intake (TDI) for patulin in a normal serving size. At 8°C, patulin was detected only in the top layer A and was not detected in all samples incubated at 4°C. In contrast, roquefortine C was produced at all temperatures, but was detected in all layers only at 15°C. The growth of *P. verrucosum* on crème fraiche was small and restricted. Ochratoxin A and citrinin were observed only in layer A at 15°C after 21 days. However, the concentration of both mycotoxins was high and corresponded to an intake above the health-based guidance values for a normal serving**.** Thus, mould growth and toxin production are delayed when food is stored at low temperatures. Despite a small colony size, substantial amounts of mycotoxins may be formed. General advice for consumers is to avoid mouldy food.

04. *Aspergillus* section *Nigri* in onions bulbs – Occurrence, identification and production of fumonisin B2 and ochratoxin A

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Fungi can spoil onions in the field and post-harvest and the deterioration may persist during transport, storage and marketing. One common disease is known as "black rot" caused by black aspergilli. The aim of this work was to isolate and identify *Aspergillus* section *Nigri* species from onion bulbs collected at different production stages and evaluate the contamination of ochratoxin A (OTA) and fumonisin B2 (FB2) in the samples. A total of 82 samples were analyzed: 40 collected from the market, 23 from field and 19 soil samples. For the fungi isolation, direct plating of 50 pieces of onion was carried out in Dichloran agar 18% Glycerol (DG18), after disinfection with sodium hypochlorite 0.4%. For soil samples, dilution plating was performed in DG18. The potential of OTA production by the species was evaluated in Yeast Extract Sucrose (YESA) medium, agar plug technique and Thin Layer Chromatography (TLC). The evaluation of FB2 production by the isolates was tested in agar Czapek 20% Sucrose (CY20S). OTA and FB2 from bulbs were analyzed using an immunoaffinity column and High Performance Liquid

Chromatography (HPLC) with a fluorescence detector. FB2 was analyzed after derivatization with o-phthaldialdehyde (OPA). In onion bulbs, the average infection by *Aspergillus* section *Nigri* was 42%, ranging from 0% to 100%, resulting in a total of 1,337 isolates. Market samples showed a higher level of *Aspergillus* section *Nigri* contamination, with average infection of 64%, comparing to field samples, with 3%. Only 21 isolates (2%) from total *Aspergillus* section *Nigri* isolates were able to produce OTA. From three hundred and sixty isolates tested for FB2 production, 53% (169) were producers. *A. welwitschiae* was the main species found in onion bulbs based on molecular approach. Onion bulbs did not show contamination by OTA and FB2.

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05. *Aspergillus welwitschiae* isolated from grapes produce ochratoxin A

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Recently, the taxon *Aspergillus niger* sensu stricto has been split into *A. niger* and *A. welwitschiae*. Both species cannot be distinguished by phenotypic or ecological data including extrolite profiles. So, this species has not yet been reported frequently because this old species name has been reintroduced not long ago. To date, there is no ecophysiological information of *A. welwitschiae*. In this study, two *A. niger* strains and five *A. welwitschiae* strains, isolated from wine grapes and raisins were studied. All the strains were previously detected as OTA producers in our laboratory and had been initially identified as *A. niger*. All the strains were confirmed for identity by sequencing of the calmodulin gene. The aim of this study was to determine the effects of water activity (aw) (0.90; 0.95 and 0.98-0.99), culture media (Yeast Extract Sucrose Broth (YESB); Synthetic Grape Juice Medium (SGM); White grape juice (WGJ)) and temperature (15 °C, 25 °C and 35 °C) on the growth and OTA production of these strains. The assay was performed in microtiter plates, determining the absorbance at 530 nm and the concentration of OTA at 1, 2, 4 and 10 days. No significant differences were observed in absorbance and OTA production values between these two species. However, in this study, we have confirmed that *A. welwitschiae* strains from wine grapes and raisins are able to produce OTA.

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0.6 Impact of interacting climate change factors on the ecophysiology, toxin gene expression and ochratoxin A production by *Aspergillus carbonarius*

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Ochratoxin A (OTA) is a potent pentaketide nephrotoxin widely contaminating food and feed products (grains, legumes, coffee, dried fruits, meat derived products, beer and wine). OTA is the primary mycotoxin risk in wine and dried vine fruits. The contamination of grapes and wine is strongly related to climatic conditions during cultivation. There is little information on the impact that climate-change induced environmental fluxes may have on fungal colonization and ochratoxin A contamination of grapes. It has been suggested that the Mediterranean region is a hot spot for the impact of climate change scenarios with temperatures expected to increase by +2-5°C and CO_2 to double or triple (400 ppm vs 800/1200 ppm) in the medium term. The northern region of Apulia, in the south of Italy, is normally considered to be an area where the risk of OTA in vineyards is relatively low. This study has examined the effect of i) increased temperature in the alternating day/night cycle (15-28°C vs 18-

34°C), ii) existing and predicted CO_2 concentrations (400 vs 1000 ppm), iii) conditions where water is freely available or drought stress is imposed (0.99 vs 0.93 a_w) in *A. carbonarius* strains (ITEM 7444, ITEM 5010 and B7), grown on grape-based matrices, by analysis of growth rate, OTA production and expression of key toxin biosynthetic genes. The results showed that in both non-stressed a_w and drought stressed conditions the effect of elevated CO_2 (1000 ppm) caused a generally positive stimulation of growth rates at both temperature cycles. Furthermore, while differences in the alternating day/night temperatures at 400 ppm and 0.99 a_w did not result in any variation in OTA production, elevated CO_2 condition (1000 ppm) was observed to stimulate OTA production especially at the 15-28°C cycle. These results were confirmed by gene expression analysis showing higher expression levels of OTA related genes at higher CO_2 concentration. This study showed for the first time that elevated CO_2 concentrations under different fluxes in day/night temperatures in the Mediterranean region may result in an increase of OTA contamination risk in the production chain, with grapes destined for wine production being less resilient under climate change scenarios.

0.7. Identification and quantification of metabolites produced by antifungal bioprotective cultures in dairy products

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In the context of a growing consumer's demand for clean label foods, antifungal cultures offer alternatives to chemical preservatives to reduce food fungal spoilage. Selected binary combinations of lactobacilli strains have recently been successfully used to inhibit *Penicillium commune* and *Mucor racemosus* in sour cream, yogurt, cheese model and semi-hard cheese. Our aim was to identify the compounds most likely involved in their antifungal activity in the 4 dairy products. Four chromatographic methods, targeting 56 antifungal compounds as well as volatile compounds, were combined. Overall, 53 antifungal compounds were detected, of which 33 were in significantly higher amount in at least one product inoculated with antifungal cultures compared to the controls. Depending on the dairy product and the antifungal culture, a cocktail of 2 to 27 compounds were present at a higher abundance in the presence of antifungal culture compared to the respective control products. Antifungal compounds were present at concentrations below their MIC and thus could act in synergy. Among them, the most commonly identified were acetic, hydroxyphenyllactic, 3-phenylpropanoic acid, 3-(4-hydroxyphenyl) propanoic, 5-oxopyrrolidine-2-carboxylic acids, diacetyl and a yet unidentified volatile compound. This extensive study contributes to improve the knowledge about the action mode of antifungal lactobacilli.

0.8. Diversity of *Fusarium* spp. and detection of seventeen mycotoxins in Brazilian brewing barley

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The genus *Fusarium* is composed of many significant plant pathogens, mycotoxin producers, and increasingly, of opportunistic human pathogens. Mycotoxin contamination in cereals is particularly important, due to the health risks and quality loss of grains, causing worldwide economic impact. Previous studies have reported that barley is susceptible to contamination by several mycotoxins, with the majority of them belonging to *Fusarium* toxins. For these reasons, the primary aim of this study was to determine the contamination of seventeen mycotoxins in brewing barley by LC-MS: aflatoxins

B_1, B_2, G_1, and G_2, fumonisins B_1 and B_2, ochratoxin A, deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxin) and "emerging" toxins (enniatins A, A_1, B, and B_1, and beauvericin). Due to the high recovery of *Fusarium* species and their toxins detected in 60 analyzed grain samples, the diversity of *Fusarium* species was performed based on RPB1 and RPB2 loci. Type A trichothecenes, aflatoxins and ochratoxin A were not detected in any of the samples, however, all of them were contaminated with at least one of the other *Fusarium* toxins (apart from type A trichothecenes). We emphasize that high incidences of type B trichothecenes, zearalenone and the previously mentioned "emerging toxins" were detected. Phylogenetic analysis demonstrated that isolated strains clustered within the *Fusarium sambucinum* species complex and the *Fusarium fujikuroi* species complex. This characterization is in accordance with the mycotoxin profile detected in the barley samples. These results highlight the importance of control strategies for *Fusarium* species and their associated toxins in barley. The co-occurrence of these toxins indicate that a thorough study based on the effects of brewing processing technologies should be conducted in order to evaluate the risks associated with co-exposure of these toxins through beer consumption.

09. The preservative propionic acid differentially affects survival of conidia and damages germ tubes of feed spoilage fungi

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The weak organic acid propionate is an important preservative in food and feed and inhibits the growth of various spoilage bacteria, yeasts and fungi, including mycotoxigenic fungi. The mode of action of this compound on fungal survival structures (conidia) and germ tubes of xerophilic feed-spoiling fungi is scarcely studied. We have isolated and identified fungal strains from nine samples of poultry feed originating from different countries using a shelf-life test and molecular methods. Xerophilic *Aspergillus* spp. were present very high predominance. We assessed the sensitivity of a panel of isolated fungi for propionic acid and evaluated the viability of treated conidia and germ tubes. MIC values were measured by means of a microtiter plate. Survival of conidia was tested after a 24-hour exposure to 31 mM propionic acid. To evaluate if propionic acid damaged germ tubes, a novel method was developed in which young biofilms of the fungi were tested for 30 min with 31 mM propionic acid in Erlenmeyer flasks using the live-dead fluorescent dye TOTO-1. The MIC values of 4.6 to 32.1 mM of these poultry-feed-specific fungi were well in the range as described in the literature. Propionic acid prevents outgrowth of conidia (spores) in a species-dependent manner. Twenty percent of *Aspergillus chevalieri* and 71% of *Penicillium lanosocoeruleum* conidia germinated after exposure. Germ tubes in a biofilm showed extensive cell death (62 to 85% of the germ tubes) already after a 30 min treatment with 31 mM propionic acid. *Penicillium* that had conidia that are more resistant, exhibited the most sensitive germ tubes. These results show that conidia are inactivated by propionic acid, but that germ tubes show a much higher sensitivity. These observations shed new light on the mode of action of this important preservative to prevent fungal contamination of feed.

10. Inactivation of stress-resistant ascospores of Eurotiales by industrial sanitizers

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Different fungi, including the genera *Aspergillus* (*Neosartorya*), *Paecilomyces* (*Byssochlamys*) and *Talaromyces*, produce ascospores that survive pasteurization treatments and are regarded as the most stress-resistant eukaryotic cells. The sensitivity of the ascospores to treatments with industrial sanitizers containing chlorine dioxide and iodine (iodophors) has never been assessed before.

In this study, we report the inactivation of dormant and activated ascospores by solutions of acidified sodium chlorite (ASC), chlorine dioxide and iodine (iodophors). The activity of the sanitizers was tested in droplet tests in which growth of fungus was evaluated after inoculation of a fixed number of ascospores. In addition, colony count experiments were done. Stereo microscopy was used to evaluate if spores formed germ tubes or not.

Ascospores of four species of Eurotiales were tested and showed clear variations in sensitivity. The most resilient species, *Talaromyces macrosporus* and *Paecilomyes variotii* (= *B. spectabilis*) survive 75 but not 200 ppm chlorine dioxide solution treatments. These species were able to survive 75 ppm iodine solution treatments, but low amounts of ascospores (100 to 1000 spores) could be inactivated after 16 h of treatment. Inactivated spores did not show any sign of germination after seven days following chlorine dioxide solution treatments as judged by microscopy, but iodine inactivation resulted in visibly distorted ascospores. Activation of the spores is important to evaluate if a spore is inactivated or (still) dormant. Our data suggest that dormant ascospores can be eradicated by the used sanitizers.

11. Occurrence of *Penicillium* species in healthy grapes for wine production

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The Small Carpathians wine region is the most extensive of the six wine regions in Slovakia. Wine growing has an old tradition here that remains to this day. A total of 22 wine producing grapes were collected from this region in 2016, 2017 and 2018. From the two vineyards were taken 13 samples of white wine grapes and 9 of blue ones. The objectives of this study were to gain more knowledge about mycobiota on grapes originating from Slovakia, with a focus on the genus *Penicillium* and its ability to produce mycotoxins in *in vitro* conditions, which was checked by a thin layer chromatography method. Direct plating of grapes on DRBC plates (Dichloran Rose Bengal Chloramphenicol) was used for analysis of surface mycobiota of grapes while surface sterilized grapes were used for endogenous mycobiota analysis. The plates were then incubated aerobically at 25 ±1 °C for 5 to 7 days in the dark. Overall, we isolated 1618 strains belonging to 15 genera of filamentous microscopic fungi from surface mycobiota of grapes. The most frequent genera were *Alternaria* (100% Fr), *Rhizopus* (82%), *Sordaria* (68%), *Epicoccum* (54%), *Botrytis* (50%), *Cladosporium* (45%) and *Penicillium* (41%). During the survey, 96 isolates belonging to 5 *Penicillium* species (*P. aurantiogriseum, P. brevicompactum, P. citrinum, P. expansum* and *P. glabrum*) were isolated and identified. The most frequently occurring *Penicillium* species of the samples were *P. expansum* (41% Fr, 94% of the isolated species). A total of 827 isolates belonging to 12 genera were obtained from endogenous mycobiota. The most frequent genera were *Alternaria* (100%), *Cladosporium* (77%) and *Penicillium* (45%). *Alternaria* was the most common genus in the surface and endogenous colonisation with an average relative density of 67%, followed by *Cladosporium* (7% exo, 13% endo) and *Penicillium* (6%). Fifty four isolates belonging to 6 *Penicillium* species (*P. citrinum, P. expansum, P. glabrum, P. griseofulvum, P. hordei* and *P. chrysogenum*) were isolated from endogenous colonisation. The selected isolates – *P. citrinum, P. expansum, P. hordei, P. chrysogenum* and *P. griseofulvum* were tested for for their toxigenic ability. Out of 85 strains, 74 % produced at least one mycotoxin as revealed by the method used here.

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12. Development and optimization of a group-specific loop-mediated isothermal amplification (LAMP) assay for the detection of patulin-producing *Penicillium* species

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Patulin is a mycotoxin of food safety concern occurring in food worldwide. Methods for a rapid, simple and accurate detection of patulin-producing fungi in and on food and food products are therefore urgently needed. In the current study, a loop-mediated isothermal amplification (LAMP) assay based on the isoepoxydon dehydrogenase (*idh*) gene of the patulin biosynthetic pathway was developed and optimized for the group-specific detection of patulin-producing *Penicillium* species. Specificity testing with purified DNA of 174 fungal strains representing 31 genera showed highly specific detection of patulin-producing species in *Penicillium*, *Byssochlamys* and *Paecilomyces*. The detection limit of the assay was 2.5 pg of purified genomic DNA of *P. expansum* per reaction. Moreover, the assay was demonstrated to detect patulin-producers when conidia were directly added to the reaction as template without any previous sample preparation with a detection limit of 1 x 10^3 conidia per reaction. The applicability of the assay was successfully tested on artificially contaminated apples and grapes requiring minimal sample preparation. A screening of grapes from the 2018 harvest from different locations in Germany showed no contamination with patulin-producers. Application of the assay to samples of apple juice, grape juice and apple puree showed that *P. expansum* was detected after artificial contamination. The developed LAMP assay is a promising and user-friendly tool for rapid diagnosis in quality control applications in the food and beverage industry.

13. Mycotoxins and toxigenic fungi in dried figs from different farming systems

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The common fig tree (*Ficus carica* L.) is a typical species of the Middle East and Mediterranean Region since it is well-adapted to high temperatures and low water regimes. Its cultivation has been traditionally subsidiary to other fruit-bearing or herbaceous plants so not too much effort has been done to improve harvest of fig fruits which is mainly based on traditional collecting of ripened fruits (≈ 30 % moisture content) from the soil once they drop from trees. This makes dried figs fruits highly vulnerable to contamination with filamentous fungi. Some of them, if the environmental conditions are propitious, may produce mycotoxins, mainly aflatoxins and ochratoxin A. So there is a need to evaluate the effect of different farming practices and drying on toxigenic fungi development and further mycotoxin production to ensure quality and safety of dried figs. The aim of this study was to analyse mycotoxins and toxigenic fungi of different samples of dried figs of the variety ´Calabacita` collected from two fields from Extremadura (Spain) with different farming systems (rainfed and irrigated practices) before (30% m.c.) and after their drying (<24% m.c) in a greenhouse. Two samplings at two different times were carried out. Samples were extracted with inmunoaffinity column and analysed by direct injection into the high-performance liquid chromatography with fluorescence detection (HPLC-FLD). In addition, the presence of potentially toxigenic moulds was also analysed by real-time PCR by using specific primers involved in mycotoxin biosynthesis. Results showed that 3 samples were contaminated with ochratoxin A and 1 with aflatoxins, concretely aflatoxins B_1, B_2 and G_2. All contaminated samples belonged to

dried fig (<24% m.c.) cultivated under a rainfed system. In addition, a high percentage of potentially ochratoxigenic and aflatoxigenic moulds was detected. Therefore, it is necessary to design strategies to control toxigenic moulds in dried figs by improving farming system practices and accelerating drying.

14. Effect of temperature on inactivation of strains of spoilage fungi during bread baking

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In this study, the inactivation kinetics of strains of Penicillium paneum and P. roqueforti were determined during bread baking. Three strains of P. paneum (PR03, PR04, and PR05) and P. roqueforti (PR06, PR11, and PR67) were used. Baking conditions were based on those used in baking industries (160 °C, 190 °C and 220 °C). The inactivation curves did not follow first-order kinetics, and as the primary model, the Weibull model was used with the fixed p-value. The t4Dvalue was also determined, and the secondary model was built using the log λ as a function of the baking temperature. Lower values of λ and t4D were obtained at 220 °C, and the values of this parameter were different ($p < 0.05$) among the three P. paneum strains at 160 °C and 220 °C. Two strains of P. roqueforti (PR06 and PR11) showed the highest values of t4D at 190 °C and 220 °C (10.2 and 8.18 min. respectively). The results of this study demonstrate that the different baking temperatures of the bread making process may result in the survival of fungi in the product, which may be vital in limiting the shelf life of these products. The quantification of fungal inactivation during bread baking is critical for the design of thermal process aiming to balance quality and microbiological stability of industrialized loaves of bread.

15. Recent developments in the (infrageneric) classification of *Aspergillus* and *Penicillium*

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The family of *Aspergillaceae* harbours various economically important genera, such as *Aspergillus*, *Penicillium* and *Monascus*. Recently, new insights in the taxonomy of those genera have led to numerous new species and name changes of existing species. Current taxonomic studies have shown that species delimitation in *Penicillium* is rather clear cut with no intergrading strains and only a few hybrids have been reported. Of course, individual characters may be overlapping, such as the size, form and ornamentation of conidia or growth and sporulation as related to temperature, but a large number of characters are non-overlapping. This is especially true concerning small molecule extrolites characters. Species are thus stable for many years (punctuated equilibria), and may then speciate under certain circumstances. However, there is one problem with the use of clear non-overlapping characters: these characters are not necessarily present in all isolates of a species or all species in a series, section, subgenus or genus. This phenomenon may be caused by horizontal transfer of genes or gene clusters or of epigenetic factors or mutation-based loss of certain gene clusters. Some of the more interesting categories are those between the genus and the species: subgenera, sections, subsections, series and subseries. Do these levels have nomenclatural status, are they predictive and should they be used formally in taxonomy? We present examples of these categories and discuss whether these categories between genus and species are useful in the taxonomy of *Penicillium*, *Aspergillus* and *Monascus*. Examples in e.g. sections *Roquefortorum*, *Sclerotiora* and *Brevicompacta* will be given.

16. Reduction of ochratoxin A in oats during roasting with reducing sugars

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Ochratoxin A (OTA) is a possible human carcinogen commonly occurring in many agricultural commodities particularly in oats and oat-based products. Since OTA is stable under most food processing conditions, it has been detected in processed food products such as breakfast cereals as well as the cereal grains. Therefore, the effect of roasting process and added sugars on the reduction of OTA in oat-based cereals were investigated. Moisture contents of oat grains were adjusted to 16% (wet weight basis, wb) and OTA was spiked at 100 μg/kg. Then the grains were roasted at 120°C and 180°C for 30 min and 60 min. The concentration of OTA in roasted oat-based cereals decreased with increasing roasting temperature and time in the ranges of 2 – 18%. Greater reduction of OTA was observed when oat grains were roasted with reducing sugars, i.e. glucose (11%) and fructose (15%), compared with oat-based cereal samples with no added sugar (10%). These results suggest that increased reduction of OTA in oats may be achieved by roasting with sugars, which can be applied to commercial production of cereal-based snack foods. In addition, known thermal degradation products such as OTA isomer, OTλ, and OTλ amide were not formed during the roasting.

17. Influence of peanut harvest date on *Aspergillus* section *Flavi* infection and fatty acid composition

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This study aimed to compare the influence of the harvest date on *Aspergillus* section *Flavi* infection and the chemical composition of two cultivars of high oleic peanut. Samples were collected from a peanut cultivation experiment carried out in the state of São Paulo. The variables analyzed were cultivars (IAC OL3 and IAC 503) both high oleic peanuts cultivars and the harvest date (two weeks earlier, one week earlier, harvest at the ideal time and one week after). Peanut samples were surface disinfected and were directly plated onto Dichloran 18% Glycerol agar. Soil samples were analysed by dilution plating. The potential for aflatoxin production was evaluated by the agar plug technique. The presence of aflatoxins in peanuts was performed using an immunoaffinity column and quantified by HPLC, reverse phase with fluorescence detection. The shelled peanut samples were graded according to kernel damages and sizes. The fatty acid composition analysis was performed using gas chromatography with a flame ionization detector (FID). *Aspergillus* section *Flavi* were present only in two samples of IAC 503 cultivar, one from harvesting one week earlier (40% of isolates were aflatoxin B producers and 60% aflatoxin non-producers) and the other one in a sample from harvest at the ideal date (33% aflatoxin non-producer, 33% aflatoxin B producers and 33% aflatoxin B and G producers). From the IAC OL3 cultivar, two samples from harvesting one week earlier were infected with *Aspergillus* section *Flavi* (50% aflatoxin non-producer, 37.5% aflatoxin B producers and 12.5% aflatoxin B and G producers). Also, all samples from harvesting one week after ideal harvest were infected (33% aflatoxin non-producer, 33% aflatoxin B producers and 33% aflatoxin B and G producers). No peanut samples showed aflatoxin contamination. The shelling percentage ranged from 69 to 73% for IAC 503 cultivar for the first and last harvest time. However, for IAC OL3 cultivar there was no variation with a percentage of 75%. The ratio of oleic to linoleic acid (O/L) ranged from 28:1 to 27:1 for IAC OL3 and from 19:1 to 27:1 for IAC 503 from the first to the last harvest date, respectively. This study was an initial step for a project that aims to predict aflatoxin production during pre-harvest and proposes different harvest dates for the aflatoxin contamination reduction. Thus, it was concluded that the cultivar IAC OL3 would allow an early harvest without causing significant changes in productivity and grain quality.

18. Antifungal capacity of phenolic acids against fungal postharvest pathogens

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Fungal growth is the main cause of fruit decay, and is usually control by the application of synthetic fungicides, however, currently their used have been restricted. Therefore, it is urgent to find alternative antifungal substances. Phenolic compounds are secondary metabolites of plants and naturally present in fruit which have been associated with antimicrobial effect. The aim was to investigate the inhibitory activity of the main phenolic acids identified in citrus peel against three molds responsible of fruit decay. The antifungal activity of ferulic acid and *p*-coumaric acid were tested *in-vitro* against *Botrytis cinerea*, *Monilia fruticola*, *Alternaria* spp. by following the ability to grow in an apricot-based medium containing concentration range from 1 mM to 15 mM. The mold growth was monitored by automated tubidimeter Bioscreen C. Overall, the inhibitory effect of ferulic acid was higher than *p*-coumaric acid against the three molds assayed. Ferulic acid showed high growth inhibition close to 100% against *M. fruticola*, *Alternaria* spp. and *B. cinerea* at concentrations of 2 mM, 3 mM and 5 mM, respectively. The antifungal effect of *p*-coumaric acid was only outstanding against *M. fruticola*, that was completely inhibited at 2 mM, whereas relevant effect against the other two fungal pathogens was at a concentration higher than 7.5 In conclusion, these results suggest the possibility of using ferulic extracts as a safer alternative to synthetic fungicides to control postharvest decay of fruit.

19. Occurrence of *Ascosphaera apis* in fermented bee pollen

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Bee pollen is known for its high content of nutritionally important substances. It is considered as the most complete food in the nature. However, due to the thick wall their digestibility is insufficient. In natural conditions, bees make nutrients available through the fermentation of the pollen in the hive. In the present work we focused on testing of different methods of pollen cans (fermented pollen) production and storage with regards to filamentous microscopic fungi (FMF) occurrence. Totally 3 types of bee pollen and 3 types of honeys of Slovak origin, water and yoghurt as a starter (in one variant) were used for the production of pollen cans. Raw materials and fermented pollen cans were subjected to mycological analyses by means of plate dilution method and DG18 (agar with dichloran and 18% of glycerol) medium was used. Samples of honey and yoghurt were free of FMF. Pollen represents the main potential source of pollen cans contamination. The highest number of fungi was found in the late spring bee pollen and *Cladosporium* spp. occurred with the highest relative density. The counts of FMF were reduced by fermentation and botanical origin of pollen had the greatest impact on their expansion. The greatest diversity of genera was recorded in bee pollen samples, subsequently declined in pollen cans has and after total fermentation was the lowest. Cans were then stored in a refrigerator for 6 months, with a mycological analysis performed after each finished month. The greatest decrease occurred after the first month of storage, later the micromycetes stabilized, respectively only slightly declined. An interesting finding was the fungus *Ascosphaera apis*, which occurred mainly in the variant where the first phase of the production took place under the access of oxygen. In two samples of this variant (with early spring bee pollen and late spring bee pollen), we detected this fungus - in one sample we recorded its occurrence until the 4th month of storage and in the second sample until the 6th (last tested) month of storage. It is interesting to note that the occurrence of this fungus was not observed

until the cans were kept in refrigerated temperatures. This fungus is known as an insect pathogen, causes chalkbrood disease in honey bees.

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20. Transcriptomic and metabolomic shifts on *Aspergillus flavus* during maize storage at different climate change environmental conditions

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There is a significant interest in the impact that climate change (CC) factors may have on mycotoxigenic fungi. In previous research, we examined the impact that 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 phenotypic aflatoxin B_1 production by *Aspergillus flavus* (NRRL3352). However, to date, there has not been any research analysing temporal shifts in the secondary metabolite production pattern of *A. flavus* under current and predicted CC conditions (water activity, a_w; 0.985, 0.93), temperature (30, 37°C) and CO_2 exposure (400, 1000 ppm).

In this work, we highlight the impact of interacting abiotic CC factors on secondary metabolite gene clusters and the related metabolome data including aflatoxin B_1 and up to 167 other fungal secondary metabolites in a kinetic study of maize after 4- and 8-days storage. Aflatoxin B_1 production increased under elevated CO_2 conditions. Similarly, metabolomic production shifts were observed for other secondary metabolites related including aflatoxin B_1 derivatives, metabolites from the aflatoxin pathway and some other metabolites during colonisation of the maize grain. This study provides in depth new knowledge using RNAseq and metabolomics analyses of the dynamics and impacts that 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.

21. Screening for detection of autochthonous yeast with antagonist activity against spoil molds from raw ewe's milk cheese

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Moulds represent one of the main spoilage microorganisms of fermented dairy products. Physicho-chemical and nutritional conditions of cheese allows the growth of filamentous fungi, in particular species from genera *Penicillium, Mucor, Phoma,* and *Fusarium*. Cheese spoilage by moulds causes important economic losses in the dairy industry and is commonly controlled by using synthetic antifungal agents. However, the emergence of fungicide-resistant spoilage microorganisms and application restrictions demand new alternative strategies. In this context, biocontrol of fungal pathogens by antagonistic microorganisms adapted to cheese environment represents a promising and eco-friendly tool. Therefore, the aim of this work was to evaluate the potential antifungal activity of autochthonous yeasts against the main spoilage mold species in raw ewe's milk cheese from Extremadura region. The antagonistic capability of 83 yeast cheese isolates was tested by challenging

the yeasts with five spoilage mould strains, *Mucor plumbeus*, *Mucor circinelloides*, *Penicillium commune*, *Phoma leveilley*, and *Fusarium verticillioides*, on milk agar at pH 5, 2% NaCl and different water activity conditions. The results obtained showed that none of the yeast strains had activity against *Penicillium commune*. However, interestingly 14 strains, 6 *Kluyveromyces lactis*, 2 *K. marxianus*, 4 *Pichia jadinii*, and 2 *Geotrichum candidum*, displayed a remarkable activity against three moulds species, *M. plumbeus*, *M. circinelloides*, and *Fusarium verticillioides*, at water activity ranging from 0.92 to 0.97. In relation to *Phoma leveilley,* a different antifungal spectrum was obtained, and the majority of strains from *Yarrowia* genera reduced its growth. In conclusion, although further investigations of the mechanisms of inhibitory activity and the effectiveness in cheese are necessary, these results indicated that specific yeast strains may be successfully applied to control mold spoilage in cheese.

22. Intraspecific variability in cardinal growth temperatures and water activities within a large diversity of *Penicillium roqueforti* strains

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The intraspecific heterogeneity of fungal growth behaviour in response to environmental factors is relevant towards decision tools such as predictive mycology but remains poorly documented. The present study investigated the intraspecific variability of cardinal temperatures and water activities (a_w), namely, minimal (T_{min} and a_{wmin}), optimal (T_{opt} and a_{wopt}) and maximal (T_{max}) temperatures and/or a_w for radial growth of 29 *Penicillium roqueforti* strains belonging to 3 genetically distinct populations. The mean values of cardinal temperatures and a_w for radial growth were significantly different among the tested strains (except for T_{max} which was constant), indicating that intraspecific variability can strongly affect predictions of radial growth. Noteworthy, absolute differences between highest and lowest values were of 22.5 °C for T_{min}, 7.6 °C for T_{opt} and 0.185 for a_{wmin}. The relationship between the intraspecific variability of the biological response to temperature and a_w and putative genetic populations (based on microsatellite markers) within the selected *P. roqueforti* strains was also investigated. Most strains used for blue cheese manufacturing displayed homogeneous growth behaviour, in comparison to strains isolated from non-cheese samples, highlighting a possible selection by cheese producers and/or by the natural conditions prevailing during cheese ripening. Overall, the present data support the idea that a better knowledge of the response to abiotic factors at an intraspecific level would be useful to model fungal growth in predictive mycology approaches.

23. Fungi and toxins in cassava: from the field to consumer

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Cassava is an amyloid root that is part of the daily diet of millions of people, especially in developing countries. Studies on cassava mycobiota, as well as the identification of fungi producing toxins, contribute to the safety of consumer health. The aims of this research were to investigate the mycobiota of cassava and its by-products and to identify the presence of potentially aflatoxin producing species. Additionally, the aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2) contamination in cassava was quantified. The presence of fungi and aflatoxins was evaluated from the field to the final product, totalling 68 samples: soil (n= 12), roots (n= 14) and cassava flours (n= 42). Direct plating with superficial disinfection and dilution plating methods were used. The potential of aflatoxigenic species was analyzed using the agar plug method and the presence of aflatoxins using High-performance Liquid Chromatography. More than 20 different genera were found, among these, *Aspergillus* spp. and *Trichoderma* spp. were

predominant, corresponding to 35.5% of total fungal incidence. *Aspergillus* section *Flavi* were isolated from 63.6% of the soil and 36.4% of the cassava root samples. Out of *Aspergillus* section *Flavi* isolates, 36.4% were producers of AFB_1, AFB_2, AFG_1 and AFG_2, 31.8% only AFB_1 and AFB_2 and 31.8% were non-producers. Although most of the isolates were toxin-producers, no samples of cassava and its by-products showed aflatoxin contamination.

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24. Effect of ZnO-Nanoparticles on *Aspergillus flavus* and *Fusarium proliferatum* growth on maize grains under environmental interacting conditions

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Maize (*Zea mays* L.) is the second most important cereal crop worldwide, after wheat, used in human and animal diets as well as raw material for food and pharmaceutical industries. In Argentina, around 60% of maize is exported and the remaining amount is used as feedstuff. *Aspergillus flavus* and *Fusarium proliferatum* are two mycotoxigenic species that frequently contaminate maize and produce aflatoxins and fumonisins, respectively. Since ZnO is a non-toxic compound used as an efficient antimicrobial agent, ZnO nanoparticles (ZnO-NPs) could be a strategy of low cost and low environmental impact to reduce mycotoxin accumulation in stored maize. The aim of the present study was: to evaluate the effect of ZnO-NPs (0, 5, 10 and 25 mM) on growth of two *A. flavus* strains (RCAF016 and RCAF018) and two *F. proliferatum* strains (ITEM 15699 and ITEM 15670) under interacting conditions of water activity (0.96, 0.97 and 0.98 for *A. flavus*, and 0.97, 0.98 and 0.995 for *F. proliferatum*) on irradiated maize grains. A concentration of 10 mM zinc acetate was included to compare their effect with the same concentration of ZnO-NPs. ZnO-NPs were synthesized according to the drop by drop mixing method and characterized by surface electron microscopy (SEM). Growth rate (mm/day) was obtained by linear regression during the linear phase of growth. It was observed that growth rates of *A. flavus* and *F. proliferatum* decreased significantly as water activity (a_W) decreased and ZnO-NPs concentration increased ($p \leq 0.05$). The percentages of growth reduction under ZnO-NPs treatments were the highest at 25 mM of ZnO-NPs. This concentration brought 39 % and 40% of growth reduction at 0.97 a_W for *A. flavus* RCAF016 and RCAF018, respectively. However, higher growth reduction percentages were observed for *F. proliferatum* ITEM 15699 (74 %) and ITEM 15670 (100%). Zinc acetate at 10 mM was less efficient than the same concentration of ZnO-NPs in reducing growth of both *A. flavus* and *F. proliferatum* showing the relevance of using ZnO-NPs.

25. Toxigenic fungi and mycotoxins in black pepper

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The aim of this study was to isolate and identify *Aspergillus* section *Flavi*, *Nigri* and *Circumdati* species from black pepper consumed in Brazil, investigate the ability of aflatoxins (AFs) and ochratoxin A (OTA) production by the isolates and assess the presence of these mycotoxins in black pepper samples. A total of 60 samples of black pepper were investigated using direct (grains) and dilution plating (powder) in Dichloran 18% Glycerol agar. The results were expressed in percentage of infection and CFU/g, respectively. Toxigenic potential was evaluated using agar plug technique and thin layer chromatography and for OTA and AFs analyses, immunoaffinity column and high-performance chromatography with fluorescence detector were used. Several species of *A.* section *Flavi* were found, among them *A. flavus*,

A. tamarii, *A. pseudotamarii* and *A. parasiticus*, totalizing 989 isolates. The frequency of occurrence of *A. flavus* was 42% in the samples with disinfection and 64.5% in the samples without disinfection, and 55% in the powder samples. Among 373 *A. flavus* isolated 38.3% were aflatoxin producers. Aflatoxins were found in 51.6% of the black pepper samples with levels varying from 0.09 to 11 µg/kg, with an average of 0.63 µg/kg. Concerning *Aspergillus* sections *Nigri* and *Circumdati* in the grain samples, the average infection was 15.4% and 1%, respectively. An increase of contamination by these species was observed in the samples without surface disinfection of 40.7% and 6%, respectively. The average contamination of the powder samples was $3.73x10^3$ CFU/g for *Aspergillus* section *Nigri* and $7.65x10^2$ CFU/g for *Aspergillus* section *Circumdati*. A total of 1,064 *Aspergillus* section *Nigri* and 132 *Aspergillus* section *Circumdati* were isolated according to their morphological and physiological characteristics, and 3.85% and 3.79% were OTA producers, respectively. The main group of OTA producers was characterized as belonging to *A. niger* aggregate (76.6%) followed by *Aspergillus* section *Circumdati* (38.3%). A total of 54 *Aspergillus* section *Nigri* representatives of total isolates were submitted to molecular identification and the following species were found: *A. niger* (19), *A. welwitschiae* (15), *A. luchuensis* (14), *A. carbonarius* (2), *A. brunneoviolaceus* (2), *A. japonicus* (1) and *A. neoniger* (1). Regarding to *Aspergillus* section *Circumdati*, 14 strains were sequenced and identified as *A. pallidofulvus* (8), *A. westerdijkiae* (3) and *A. ochraceus* (3). Ochratoxin A was found in 55% of the samples but in general at low levels, ranging from 0.05 to 13.15µg/kg (average of 1 µg/kg). Although there was a high incidence of ochratoxigenic fungi, the levels of ochratoxin A found in the black pepper samples were below the established limits of Brazil and the European Union.

26. Development of new avoidance strategies for aflatoxin contamination in maize grains in the framework of AflaZ, a new project to control aflatoxins in food

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Food and feed contaminated by filamentous fungi have become an extensive problem. In addition to allergies caused by mold, the production of mycotoxins is a serious health risk. Some *Aspergillus* strains of the species *A. flavus* and *A. parasiticus* are able to produce aflatoxins which are toxic and carcinogenic to humans and animals. A major problem is the contamination of cereals by *Aspergillus* in subtropical countries, as the climate benefits mold growth and the technical possibilities for the reduction of mycotoxin contaminations are still limited. However, the environmental conditions that lead to a high production of aflatoxins should be comprehensively analysed with respect to influences associated with climate change. The international research cooperation project AflaZ (Z = zero aflatoxin) between German and African Research institutions, is therefore intended to significantly contribute to the elucidation of aflatoxin formation and the development of suitable avoidance strategies. Since the production of aflatoxins occurs mainly after harvesting, suitable storage conditions must be analysed. In order to develop sustainable and cost-effective avoidance strategies, toxigenic strains of *Aspergillus* are cultured under variable physiological conditions. First results show that the two aflatoxin producing species, *A. flavus* and *A. parasiticus,* show a completely different response to physico-chemical influence factors such as light of different wavelength, temperature and substrate composition.

27. Volatile organic compounds produced by yeasts inhibit mycotoxin production by postharvest pathogenic moulds

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Some fruits are highly susceptible to fungal contamination with saprophytes at the postharvest stage which may result in economic losses for producers. Some of them are toxigenic species which may produce mycotoxins, poisonous compounds which pose a risk to consumers' health so that control of toxigenic fungi contamination is needed. Use of biocontrol strategies, specifically yeasts, is an emergent strategy to control mycotoxins in fruits. It has been described that yeasts may have different modes of antagonistic activity against toxigenic moulds including competition for nutrients and space, blockage of mycotoxin-related genes or the production of extra-cellular compounds such as volatile organic compounds (VOCs). The objectives of this study were to: a) evaluate the efficacy of VOCs produced by two yeast strains (*Hanseniaspora uvarum* L793 and *Hanseniaspora opuntiae* L479) to inhibit the growth of some postharvest pathogenic moulds and their mycotoxins (aflatoxins and patulin) production and, b) identify the VOCs producers of the inhibitory activity. In addition, the effect of VOCs on the expression of genes involved in mycotoxin biosynthesis was also examined. For this, a double agar plate technique containing PDA was used. The top plate was inoculated with yeast cells and the bottom plate with spore suspensions of the mould strains and incubated at 20ºC for at least 21 days. Mould growth and mycotoxin quantification were determined at the end of the incubation period. Control samples without the presence of yeasts were also analysed. VOCs produced by both yeasts were extracted by SPME and analysed by gas chromatography. Results showed that VOCs produced by *H. uvarum* L793 produced a great inhibition of mould growth and aflatoxin production. Two of the most active antagonistic VOCs compounds were 1-butanol 3-methyl acetate and 2-phenyl ethyl ester. In addition, it was observed that VOCs affected mycotoxin-related gene expression. The implications for these results are discussed.

28. Mycobiota and mycotoxin occurrence in chickpea produced in Argentina

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Chickpea (*Cicer arietinum*) is one of the most cultivated pulses in terms of world production. There is a high demand of this legume due to its nutritional value. Although it is more popular in developing countries, it is becoming recognized throughout the world. Chickpea is often attacked by fungi during pre and post-harvest stages, significantly affecting its productivity. Also some species can be potential mycotoxin producers that can lead to serious threats to human health. Since there is an increasing demand for high quality and innocuous foods, limits for mycotoxin contamination have been established. The aims of this survey were to determinate mycobiota and mycotoxin contamination in chickpea seed samples harvested from different chickpea growing areas in Argentina during the 2018 harvest season. All samples showed contamination with at least one fungal genus. In general, infection levels ranged from 10 to 100%. The most prevalent fungal genera isolated were *Aspergillus* and *Alternaria*. Other fungal genera isolated in less frequency were: *Penicillium, Chaetomium, Rhizopus* and *Fusarium*. Mycotoxin contamination was analyzed in 10 chickpea samples by LC-MS/MS. Although *Fusarium* was not the predominant fungal genus isolated, most detected mycotoxins were

those produced by members of this genus. As a result, deoxynivalenol, zearalenone, beauvericin and alternariol monomethyl ether were detected in all samples, in levels ranging from 26.1 - 626.2 ng/g, 1.71 – 227.1 ng/g, 7.5 – 73.7 ng/g and 0.7 – 14.5 ng/g, respectively. In 40% of analyzed samples, 3-acetyldeoxynivalenol was found in levels ranging from 12.7 - 50.744 ng/g. Alternariol was detected in 30% of samples in levels ranging from 1.4 - 2.3 ng/g. Only one sample was contaminated with fumonisins (16.4 ng/g and 15.3 ng/g for FB_1 and FB_2, respectively). Another sample was contaminated with 20.5 ng/g of 15- acetyldeoxynivalenol. The occurrence of *Fusarium* mycotoxins at harvest time could indicate that *Fusarium* contamination occurs under field conditions during grain development, when high water activity levels are observed.

29. Predictive models for quantitative assessment of antifungal activity of nanoparticles (NPs)

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Metal nanoparticles represent a potential alternative solution to prevent fungal proliferation in foodstuffs since they can effectively act as fungicidal agents for example in packaging material, or air filter coatings. While different experimental procedures for the antifungal activity assessments of metal nanoparticles are available, more quantitative data, including safety limits and effective concentrations, should still be collected and analysed by predictive modelling tools. The application of the different modelling tools is dependent on the experimental designs and the techniques used to assess effectively the antifungal activity of nanoparticles at a microscopic and macroscopic level. This work reviews predictive models suitable for quantitative assessment of antifungal activity of metal NPs. On one hand, regression methods to correlate diameters/radius of colonies with time at different concentrations of NPs are applied to estimate the growth rate of specific fungi. Data can then be further processed by estimating the growth rates per each NPs' concentration. On the other hand, when the focus is on the estimation of the minimum inhibitory concentration (MIC) and the non-inhibitory concentration (NIC) of NPs, the quantification of the fractional areas as reported from turbidimetric measurements can be applied. Analysis by a polynomial logistic regression describing the probability of growth/no growth in the presence of different NPs concentrations can also be effective when performing experiments on agar disk diffusion methods. Recent quantitative methods also include the evaluation of the percentage of germination of spores over the total spores which can assess the germination kinetics, i.e., slope and geometrical lag time of single spores, in presence of NPs. In conclusion, the importance of selecting appropriate modelling structures for estimating accurate and precise parameters that assess the efficacy of antifungal compounds is an important iterative procedure.

30. Heat-resistance of *Humicola fuscoatra* and *Talaromyces wortmannii*, two fungal species contaminating industrial packaging materials

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Contamination of packaging materials used by food industries has gained attention in recent years, due to the increasingly widespread use of holistic approaches in considering problems linked to food spoilage by microorganisms. When background or incidental spoilage cases by Filamentous Fungi occur, the search for the responsible organisms must include also Heat Resistant Moulds (HRM), since they are usually associated with raw materials, but they have been recently found in packaging

materials (e.g. plastic caps and their boxes, PET bottles, slip sheets, layer boards, palletizers, etc.) and processing environments, too.

This work has been carried out to evaluate the thermal resistance of two fungal species, *Humicola fuscoatra* and *Talaromyces wortmannii,* isolated at the SSICA from some of the above-mentioned materials at concentrations up to 85 CFU/cm^2. Since no literature data about their heat-resistance have been released so far and a transfer from packaging materials to finished products could be supposed, it could be of great importance to collect data about their inactivation. *H. fuscoatra* proved to possess high decimal reduction values (D_{80}=76'; D_{84}=30'; D_{88}=16'; D_{92}=9' in a glucose solution; D_{80}=42'; D_{84}=20'; D_{88}=10'; D_{92}=6'in blueberry and grape juice), its parameters being incompatible with those industrially applied during the sanitization of the packaging by heat of a non-aseptic filling process. On the contrary, *T. wortmannii* proved markedly less heat-resistant (D_{71}=8.2'; D_{75}=1.4'; D_{78}=0.3' in a glucose solution; D_{71}=7.0'; D_{75}=1.4'; D_{88}=0.3' in blueberry and grape juice), its inactivation being therefore possible within a traditional industrial hot-filling process. These data have stressed the importance of a continuous monitoring of HRM in packaging materials, since they proved to be a potential source of contamination and a cause of background spoilages for acid products.

31. Inhibition of the aflatoxin biosynthesis of *A. flavus* by arginine

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The contamination of maize with mycotoxins, like aflatoxins produced by *Aspergillus flavus*, poses a serious health risk in sub-Saharan countries and will most likely be an increasing problem in European countries in the future due to climate change. One goal of the ongoing collaborative project AflaZ is the reduction of aflatoxin contamination in sub-Saharan countries to improve the food safety among others by finding natural inhibitors of the growth of *A. flavus* and of its mycotoxin production. A variety of amino acids can influence the biosynthesis of mycotoxins of different fungi. According to literature data it has been shown that the amino acid arginine has a quite broad spectrum of inhibitory activity against various mycotoxin biosynthetic fungi. The objective of this study was to investigate the effect of arginine on the aflatoxin biosynthesis of *A. flavus* grown on maize medium at 25°C for 7 days. The fungal growth was reduced starting with 20 mM arginine added to the medium, whereas a complete inhibition was achieved by adding 80 mM arginine. Additionally, a reduction of the aflatoxin production was measured. These results were independent of the pH value of the medium. Interestingly, it was shown that the production of cyclopiazonic acid as a further mycotoxin increased by adding arginine and that the regulation of its production depends on the pH value. The exact mechanism explaining the effect of arginine on the mycotoxin production of *A. flavus* still has to be clarified. However, these results question if aflatoxin and cyclopiazonic acid are regulated by the same molecular mechanism as it is proposed in the literature.

32. Analysis of genetic diversity in the *Aspergillus niger* clade

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The aim of this study was to investigate the genetic diversity of the *Aspergillus niger* clade. Firstly, we investigated the molecular diversity using calmodulin gene (*CaM*) sequences of approximately 700 accessions belonging to this clade. Based on *CaM* sequences, eight haplotypes were clearly identified as *A. niger* (*n*= 247), and 17 were identified as *A. welwitschiae* (*n*= 403). However, *CaM* sequences did not provide definitive species identities for six haplotypes, representing 45 strains. To elucidate the taxonomic position of these haplotypes, two other *loci*, part of the beta-tubulin gene and part of the

RNA polymerase II gene, were sequenced and used to perform an analysis of Genealogical Concordance Phylogenetic Species Recognition. In addition to *A. niger* and *A. welwitschiae*, this analysis suggested the recognition of two other phylogenetic species. *The strains representing one of the new* phylogenetic species *were found morphologically* distinct in relation to *A. niger* and *A. welwitschiae* strains, mainly as follows: a) reduced growth *on MEA medium*; b) white mycelial area with sparse sporulation on CYA medium; and c) large globose to ovoid cream-coloured sclerotia produced on CYA agar. Analyses by Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) are ongoing to identify the secondary metabolites. Concluding, when the results of Genealogical Concordance Phylogenetic Species Recognition and morphological evidences are jointly considered, our study suggests that a further dismemberment of the *A. niger* clade is required.

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33. The effect of selected essential oils on the growth of *Botrytis cinerea*

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The aim of this research was to determine the influence of selected essential oils from plant family *Lamiaceae* (basil, rosemary, peppermint, savory, and sage) and from family *Lauraceae* (cinnamon, laurel leaf, may chang, camphor) on the growth of three isolates of *Botrytis cinerea*. Isolates were acquired from mouldy strawberries. The antifungal activity was evaluated by the modified micro-atmosphere method. The strains were inoculated in the middle of Petri dishes. Sterile filter paper was placed in the center of the Petri dish and the essential oil (EO) at the concentration 625 µL essential oil.L^{-1} of air was applied. Dimetylsulfoxid (DMSO) was used as control. Dishes were tightly sealed with parafilm and incubated for 7 days at 22 ±1 °C. The diameters (Ø mm) of the growing colonies (from the reverse side) were measured at the 2nd, 3rd, 4th and 7th day with a digital calliper. Essential oils that completely inhibit the growth of all strains (peppermint, savory, sage, cinnamon, and may change) were used to determine their minimum inhibitory doses (MIDs). EOs, dissolved in DMSO, were prepared at different concentrations (500, 250, 125, 63, 31.25, and 15.63 µL.L^{-1} of air). Cultivation was carried out at the 22 ± 1 °C. MID was expressed as the lowest concentration of essential oil in which no visible growth of the test strains was observed after 7 and 14 days of culture compared to the control sample. Using probit analysis, predicted MIDs90 and MIDs50 were calculated. All tested essential oils markedly affected the growth of isolates of *B. cinerea* in order: may chang (*Litsea cubeba* (Lour.) Pers) > peppermint (*Mentha x piperita* L.) > savory (*Satureia hortensis* L.) > cinnamon (*Cinnamomum zeylanicum* Blum) > sage (*Salvia officinalis* L.) > basil (*Oscimum basilicum* L.) > rosemary (*Rosmarinus officinalis* L.) > laurel leaf (*Laurus nobilis* L.) > camphor (red) (*Cinnamomum camphora* L.).

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34. Difference in local pre-harvest practices linked with the occurrence of Fusarium species in maize grown by two farmer groups in Vietnam's central highlands

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Ethnic groups often apply different crop management practices. However, the impact of these different

management practices on plant pathogens has never been investigated. Therefore, we investigated the impact of different pre-harvest practices of two ethnic groups of Vietnam on the occurrence of *Fusarium* species in maize. Field maize samples were collected from Kinh farmers (Dak Nong province) and Ede farmers (Dak Lak province) in autumn-winter (AW) crop 2017 and summer-autumn (SA) crop 2018. Beside a questionnaire on agricultural practices, a detailed survey was conducted on occurrence of *Fusarium* species and their mycotoxins in each household. The data indicated that the incidence of *Fusarium verticillioides* and *Fusarium proliferatum* in maize grains grown by Ede farmers was much higher than in maize grains grown by Kinh farmers. Especially, in the SA 2018, 61.11 % of the Ede's maize grains was contaminated with *F. verticillioides*, while this fungus was found in only 22.22 % of the Kinh's maize grains. Interestingly, notwithstanding we consider weather as a confounding factor in our analysis, it was remarkable that differences in *Fusarium* occurrence correlated with pre-harvest practices, such as tillage practices, soil fertility, and crop residue management.

35. An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins

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Over an eight-year period, 686 winter 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 as well as the abundance of *F. graminearum* (FG) and *F. poae* (FP) DNA and three chemotypes, 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON) and nivalenol (NIV). Of all *Fusarium* species, FG and FP were predominant, and the average abundance of the FG DNA was three times higher compared with that of FP. In addition, the average detection of the 15ADON chemotype was twice as high as those of 3ADON and NIV, respectively. Deoxynivalenol (DON), zearalenone (ZEA) and nivalenol (NIV) were the most frequently detected toxins. For DON, 11% and for ZEA, 7% of all samples exceeded the European maximum limits for unprocessed cereals (1). Furthermore, NIV was most likely produced by four different *Fusarium* species, including FP, FG as well as *F. cerealis* and *F. culmorum*. A multiple correspondence analysis revealed that high levels of FG and DON were mainly observed in grain samples from fields with the previous crop maize, reduced tillage, cultivars with poor FHB resistance and strobilurin-based fungicides. Other previous crops and/or ploughing decreased the DON content by 78 to 95%. ZEA showed a similar pattern. In contrast, high levels of FP and NIV were associated with samples from ploughed fields and the previous crop canola (2). These findings and the negative correlations between FP DNA and FG incidence, ZEA and DON suggest a different ecological niche for FP or diverging requirements for infection. Moreover, the effect of cropping factors on FG infection and DON contamination in wheat was quantified to develop the forecasting system FusaProg. This internet-based system employs plot-specific cropping, growth stage and regional weather data (3) and was successfully validated with more than 600 wheat samples. FusaProg showed to be a highly valuable tool for targeted fungicide application and production of safe wheat.

References

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