EFFECT OF OXIDATIVE STRESS AND ANTIOXIDANT COMPONENTS ON TRICHOTHECENE B ACCUMULATION AND ON *TRI* GENE EXPRESSION IN *FUSARIUM* SPECIES GROWN *IN VITRO*. **C. Barreau, N. Ponts, A.L. Boutigny, L. Pinson-Gadais, V. Atanasova and F. Richard-Forget.** *Institut National de la Recherche Agronomique, UR1264 MycSA, BP81, Avenue Edouard Bourleaux, 33883 Villenave d'Ornon, France. E-mail: cbarreau@bordeaux.inra.fr*

Although the genes involved in the biosynthesis of trichothecenes B have been described for *Fusarium graminearum*, it is not clearly known how a high level of toxin production is induced. Accumulation of the toxin is highly dependent of the substrate *in vitro*. *In planta*, the substrate available for the fungus may vary greatly depending of the plant host (differences in grain biochemical composition) and on the way the plant reacts (defence mechanisms) to *Fusarium* invasion. The generation of reactive oxygen species is the earliest response. We investigated the *in vitro* effect of H_2O_2 treatments on accumulation of trichothecenes B by *F. graminearum*. Reversely, the effect of depletion of H_2O_2 by catalase treatment was also analysed. Results show that toxin accumulation is greatly modulated by the oxidative status of the medium. Effect of change in the grain composition was investigated by looking at molecules with anti-oxidant properties. Wheat grain contains a high level of phenolic acids. We addressed the question whether the biosynthesis of trichothecenes is modulated when phenolic acids are supplied to *F. culmorum* strains in a synthetic culture medium and showed that cinnamic derived phenolics with high anti-oxidant potential inhibited significantly the production of the toxin. The redox potential seems to greatly modulate the accumulation level of trichothecenes B. Effect on expression of various *TRI* genes was assessed by Q-RT-PCR. The result showed that in all the case, the modulations observed in accumulation of the toxin can be explained by a regulation at the transcriptional level.

FORMATION OF DEOXYNIVALENOL-3-*O*-GLUCOSIDE IN *SOLANUM TUBEROSUM* CULTIVARS INFECTED WITH ENGINEERED *FUSARIUM SAMBUCINUM*. **A. Bucsich[1], R. Mitterbauer[1], M. Sulyok[2], F. Berthiller[2], H. Persak[1], G. Wiesenberger[1], R. Krska[2] and G. Adam[1].** *[1]Institute of Applied Genetics and Cell Biology, BOKU – University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria. [2]Center for Analytical Chemistry and Christion Doppler Laboratory for Mycotoxin Research, Department of Agrobiotechnology, IFA Tulln, BOKU – University of Natural Resources and Applied Life Sciences, Konrad Lorenz Strasse 20, A-3430 Tulln, Austria. E-mail: gerhard.adam@boku.ac.at*

Structurally diverse trichothecenes are produced by *Fusarium* species. A role as defense suppressing virulence factors is likely, but difficult to demonstrate, since the effect may be masked by other compounds, and plants may be able to antagonize toxins to a variable extent. Structural variants of trichothecenes escaping detoxification may provide a selective advantage, which could be the driving force for trichothecene diversity. *Fusarium sambucinum* (*F. sulphureum* MRC514) was originally isolated from potato and produces the type A trichothecene diacetoxyscirpenol. It is most likely a natural *tri1* mutant, as no T-2 toxin is formed. We have converted this strain into a type B trichothecene producer. Disruption of *TRI13* led to accumulation of calonectrin and 15-decalonectrin. After transformation of the *tri13* strain with the *F. graminearum FgTRI1*, DON production in liquid Czapek was observed (up to 300 mg/l and additionally 120 mg/l 15-Acetyl-DON). Several transformants were used to inoculate surface sterilized tubers (and autoclaved tubers) of different potato cultivars. Interestingly, DON and DON-3-O-glucoside (D3G) was found in the infected tissue of living potatoes. Transformant IAJK8 produced on cultivar "Franceline" 1380 µg/l DON and 4.6 µg/l D3G, on cultivar "Ditta" 58 µg/l DON and 307 µg/l D3G. Clear differences in the ability to detoxify DON (and in the ability to reduce the spread of the engineered *Fusarium*) exist in potato. D3G is a masked mycotoxin present in wheat, but currently not readily available. Attempts to engineer D3G production by transformation of the DON producing strain with a cloned *Arabidopsis* DON-glucosyltransferase will be reported.

NIVALENOL ACCUMULATION IN POTATO TUBERS AFFECTED WITH DON-PRODUCING STRAINS OF *FUSARIUM GRAMINEARUM*. **J.A. Delgado[1], P.B. Schwarz[2], J. Gillespie[2], V.V. Rivera[1] and G.A. Secor[1].** *[1]Department of Plant Pathology, North Dakota State University, Fargo, ND, USA. [2]Departement of Plant Sciences, North Dakota State University, Fargo, ND, USA. E-mail: gary.secor@ndsu.edu*

Small grains infected by *Fusarium graminearum* are frequently contaminated with deoxynivalenol (DON) in North America, South America and Europe, but rarely with nivalenol (NIV). NIV was shown to be less toxic than DON in *Arabidopsis thaliana* leaves, but a mixture of trichothecenes has synergetic effects. Our objective was to compare the chemotype of *F. graminearum* isolates with their DON/NIV profile in dry rotted tissue of potato tubers inoculated with *F. graminearum*. The chemotype of fourteen isolates of *F. graminearum* isolated from potato, sugar beet, and wheat was assessed by GC-MS analysis of rice cultures, and by PCR assays. Twelve isolates were classified as DON-chemotype, and two as NIV-chemotype. The NIV-producing strains showed only accumulation of NIV. However, the DON-producing strains showed accumulation of both DON and NIV. Some isolates showed more accumulation of DON than NIV, whereas others showed more accumulation of NIV than DON. A TLC analysis of the same crude extract of the potato rotted tissue was performed confirming the presence of NIV along with DON. A, negative correlation between the severity of the disease and NIV production in the rotted tissue was found ($P < 0.0001$). In a preliminary study, cooked and uncooked potato slices inoculated with DON-producing strains of *F. graminearum* showed the production of DON and NIV in the uncooked slices, but only DON in the cooked slices. We believe that the accumulation of NIV in the rotted tissue of potato tubers might be due to the enzymatic interaction between the pathogen and the host.

***FUSARIUM GRAMINEARUM* MYCOTOXINS IN SEEMINGLY HEALTHY TISSUE OF POTATO TUBERS AFFECTED WITH DRY ROT.** **J.A. Delgado[1], P.B. Schwarz[2], N.C. Gudmestad[1], J. Gillespie[2], V.V. Rivera[1] and G.A. Secor[1].** *[1]Department of Plant Pathology North Dakota State University, Fargo, ND, USA. [2]Departement of Plant Sciences, North Dakota State University, Fargo, ND, USA. E-mail: gary.secor@ndsu.edu*

Potato dry rot is mainly caused by *Fusarium sambucinum*. However, the first incidence of *F. graminearum* causing potato dry rot was reported in 2005 in North Dakota and Minnesota, USA. This fungus causes the accumulation of the mycotoxin deoxynivalenol in wheat and barley grains. The objective of this study was to determine the presence and distribution of *Fusarium* mycotoxins in healthy tissue of potato tubers affected by dry rot

caused by *F. graminearum*. Mycotoxin analysis was performed by GC/MS in twenty tubers cv. Russet Burbank naturally infected and twenty tubers inoculated with *F. graminearum*. All tubers were incubated for four weeks at room temperature. The mycotoxins deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and zearalenone were detected at 1, 2, and 3 cm from the lesion margin and in apparently healthy tissue. Deoxynivalenol was found at the highest frequency and concentration. In naturally infected potatoes, deoxynivalenol was detected in 90%, 75%, and 50% of the potato tubers at 1, 2, and 3 cm from the lesion; with an average of 1.39, 0.65, and 0.16 ppm, respectively. In artificially infected potato tubers, deoxynivalenol was detected in 100%, 95%, and 40% of the potato tubers at 1, 2, and 3 cm from the lesion; with an average of 2.29, 1.22, and 0.09 ppm, respectively. These preliminary results show that *F. graminearum* produces mycotoxins in potato tubers affected with dry rot caused by *F. graminearum* that are able to diffuse at least short distances into healthy tissue when tubers are stored at room temperature.

TOXIGENIC POTENTIAL OF SOME *FUSARIUM CULMORUM* AND *F. GRAMINEARUM* STRAINS ISOLATED FROM EGYPTIAN WHEAT. Y. Gherbawy. *Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia. E-mail: youssufgherbawy@yahoo.com*

Genetic analysis of plant pathogenic populations is fundamental to the understanding of epidemiology, host-pathogen coevolution, and resistance management. To this aim, 28 strains of *Fusarium culmorum* and *F. graminearum* (14 strains each) isolated from Egyptian wheat were used to estimate the DNA similarities and mycotoxin production. The intergenic sequences of *Tri5* and *Tri6* genes involved in the mycotoxin pathways of *Fusarium* species were used for detecting deoxynivalenol (DON)-chemotypes and nivalenol (NIV)-chemotypes of *F. graminearum*, while *Tri7* and *Tri13* were used to determine the ability of *F. culmorum* strains to produce deoxynivalenol and nivalenol. Eleven and sixteen strains, respectively, were able to produced DON and NIV, while one strain was able to produce both types of mycotoxins.

OCCURRENCE OF TRICHOTHECENE MYCOTOXINS IN TUNISIAN *FUSARIUM* SPECIES ISOLATED FROM WHEAT. G.L. Kammoun[1], C. Barreau[2], F. Richard-Forget[2], S. Gargouri[1] and M.R. Hajlaoui[1]. *[1]Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, rue Hédi Karray, 2049 Tunis, Tunisia. [2]Institut National de la Recherche Agronomique, Centre de Recherche de Bordeaux, UR1264 MycSA, 33883 Villenave d'Ornon, France. E-mail: lobna_kammoun@yahoo.fr*

Fusarium Head Blight (FHB) is a disease of wheat restricted to Northern Tunisia, when high rainfalls occur during flowering stage. The most serious effect of FHB is the grain contamination with mycotoxins produced by *Fusarium* species. The predominant mycotoxins produced on cereals are type B trichothecenes deoxinivalenol (DON) and nivalenol (NIV). The aim of this study was to analyse trichothecenes produced by 30 *Fusarium* spp. (28 *F. culmorum* and 2 *F. pseudograminearum*) isolated from wheat heads in 7 various fields located in Northern Tunisia. All isolates were characterized morphologically according to macroscopic and microscopic aspect. Physiological characterisation was based on their ability to produce type B trichothecenes (deoxynivalenol, nivalenol and 3-15 acetyldeoxynivalenol) in wheat samples using high pressure liquid chromatography (HPLC) with ultraviolet detection. Trichothecenes produced by these species was analysed and then compared with those obtained after incubation in liquid culture (GYEP) and in culture on wheat. The results indicate that all strains produced DON in GYEP. The DON levels found for *F. culmorum* isolates ranged from trace to 1721.46 µg/g, while *F. pseudograminearum* isolates did not show this ability. All strains also produced DON in culture on wheat: 9.49 µg/g to 604.50 µg/g for *F. culmorum*, and 35.80 µg/g to 521.39 µg/g for *F. pseudograminearum*. The variability in the level of toxin production depends on both the cultural medium and the isolate. Differences in mycotoxin production in liquid medium or on grain suggest a role of the substrate: kernel biochemical composition may be an important factor controlling the level of induction of toxin biosynthesis.

CYTOTOXICITIES OF ENNIATINS H, I, AND MK1688 FROM *FUSARIUM OXYSPORUM* KFCC 11363P. H.S. Lee, H.H. Song, S.N. Bang and C. Lee. *Department of Food Science and Technology, BET Research Institute, Chung-Ang University, Ansung, 456-756, Korea. E-mail: chanlee@cau.ac.kr*

Enniatins (ENs) H, I, and MK1688 and beauvericin (BEA) were purified from concentrated chloroform extracts of *Fusarium oxysporum* KFCC 11363P submerged cultures using HPLC, and their *in vitro* cytotoxicities were evaluated against four human carcinoma cell lines (lung, A549; ovarian, SK-OV-3; skin melanoma, SK-MEL-2; and colon, HCT15) using the SRB method. ENs I and MK1688 inhibited the growth of cancer cell lines most strongly and similar cytotoxic effects on the tested human cancer cell cultures. The cytotoxicity of ENs I and MK1688 was three- to fourfold higher than that of BEA and EN H. When cultivated in *Fusarium* defined medium, the concentrations of ENs and BEA produced in *F. oxysporum* KFCC 11363P decreased in the following order: EN MK1688 (0.81 g/l) > EN I (0.55 g/l) > BEA (0.17 g/l) > EN H (0.16 g/l). This study has shown that ENs H, I, and MK1688 exhibit cytotoxicity against certain adenocarcinoma cell lines. The results indicate the need for more investigations into the significance of the biological properties of these new ENs.

ECOPHYSIOLOGY OF *FUSARIUM CULMORUM* AND *F. GRAMINEARUM*: EFFECT OF ENVIRONMENTAL CONDITIONS ON TRICHOTHECENE GENE EXPRESSION USING A TOXIN GENE ARRAY. N. Magan[1], M. Heydt-Schmidt[2] and R. Geisen[2]. *[1]Applied Mycology Group, Cranfield Health, Cranfield University, Bedford MK43 0AL, U.K. [2]Federal Research Centre for Nutrition and Food, Karlsruhe, Germany. E-mail: n.magan@cranfield.ac.uk*

The production of mycotoxins such as trichothecenes are predominantly dependent on nutritional matrices, and interacting environmental factors such as temperature, pH and water availability. Ecophysiological studies have determined the profiles for germination, growth and mycotoxin production to enable predictive modelling approaches to be developed. Often under environmental stress, growth is unrelated to levels of mycotoxin production. Sometimes growth inhibition occurs while significant toxin production occurs, especially under fluctuating environmental conditions. The relationship between such abiotic factors, growth and the triggering of toxin gene cluster expression need to be related to phenotypic toxin production. We have examined the impact of interacting environmental factors of water availability and temper-

ature on trichothecene and correlated expression with phenotypic DON and NIV production. The efficacy of interacting abiotic stresses on gene clusters (e.g. *tri* genes; *F. culmorum, F. graminearum)* were quantified and those being up and down regulated determined. These were correlated with phenotypic expression of DON and NIV (*F. culmorum* only). This now provides tools for targeted approaches to evaluate and test control systems directly on these toxin gene clusters under simulated environmental regimes. These targeted arrays will enable a better understanding of the influence of interacting abiotic and plant factors on regulation of important toxin gene clusters and be an excellent tool for examining novel chemical and non-chemical control strategies for prevention of mycotoxins entering the food chain.

FUSARIUM **MYCOTOXINS IN LITHUANIAN CEREALS FROM THE 2003-2007 HARVESTS. A. Mankeviciene, S. Suproniene and Z. Dabkevicius.** *Department of Plant Pathology and Protection, Lithuanian Institute of Agriculture, 58344 Akademija, K_dainiai district, Lithuania. E-mail: audre@lzi.lt*

During the period 2003-2007 spring and winter cereal grain samples were analysed for the presence of mycotoxins deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin, by ELISA (enzyme-linked immunosorbent assay) at the Lithuanian Institute of Agriculture. DON is the most common toxin produced by *Fusarium* genus found in Lithuania-grown cereal grain. It was identified in 88.4-98.3 % of the cereal grain samples assayed during 2003-2007. Spring cereals were found to be more heavily infested by this toxin than winter cereals. ZEN was more prevalent in the grain of spring wheat, barley and oats (82.9, 67.5 and 82.4%, respectively). Higher risk of zearalenon contamination was revealed in spring barley grain samples. T-2 toxin contaminated 64.7-100% of the spring and winter cereal samples tested. Higher T-2 toxin contamination levels were identified in the samples of rye (93.3%), spring barley (84.9%), and oats (100%). T-2 toxin control in Lithuania-grown grain is of great relevance, because climate conditions are conducive to the development of its producers. The most frequent T-2 toxin producers in Lithuania-grown grain of various cereal species were found to be *F. poae, F. langsethiae* and *F. sporotrichioides.* The incidence of the main producers of DON and ZEN (*F. culmorum* and *F. graminearum)* was lower, therefore the toxin concentrations in the tested samples were not high and did not exceed the levels specified in the EU regulations.

COMPARATIVE EFFECTS OF TEMPERATURE, SOLUTE AND MATRIC POTENTIAL STRESS ON GROWTH AND *FUM1* GENE EXPRESSION IN *FUSARIUM PROLIFERATUM* AND *FUSARIUM VERTICILLIOIDES*. P. Marín[1], M. Jurado[1], N. Magan[2], C. Vázquez[3] and M.T. González-Jaén[1]. *[1]Department of Genetics University Complutense Madrid, Jose Antonio Novais 2, 28040 Madrid, Spain. [2]Applied Mycology Group, Cranfield Health, Cranfield University, Silsoe, Bedford MK45 4DT, U.K. [3]Department of Microbiology III, University Complutense Madrid, Jose Antonio Novais 2, 28040 Madrid, Spain. E-mail: tegonja@bio.ucm.es*

Fusarium verticilloides and *Fusarium proliferatum* are considered the most important fungal species capable to produce fumonisins when colonize plant hosts and they may occur simultaneously in maize. The occurrence of fungal species and their mycotoxin production are influenced by ecophysiological factors, in particular water stress and temperature. The objective of this work was to study the effect of water stress, solute and matric potential, on growth rate and fumonisin gene expression in both species. The effect of ionic and non-ionic solute water stress and matric potential was analysed by measuring *in vitro* mycelial growth rates. The expression of *FUM1* gene, involved in fumonisin biosynthesis, was quantified by species-specific Real Time RT-PCR protocols. The results obtained indicated a similar general effect of water stress on growth rates, although significant differences were observed. Water stress reduced fungal growth, in particular when caused by ionic solute, matric potential and low temperature in both species, but the negative effect of matric potential stress was higher in the case of *F. proliferatum.* The temporal kinetic performed to examine the effect of ionic solute stress on growth and *FUM1* expression suggested that water stress might be a critical factor affecting fumonisin accumulation in natural conditions by both *F. proliferatum* and *F. verticillioides*, when water stress is progressively increasing during kernel maturation, in particular in the case of *F. verticillioides* which showed an increasing *FUM1* gene expression at higher water stress values and long exposure times, also suggesting differences in the regulation of *FUM1* expression between these closely related species. *F. proliferatum* may represent a similar risk for fumonisin production than *F. verticillioides* in natural conditions.

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BEAUVERICIN PRODUCTION DIFFERS BETWEEN GROUP 1 AND 2 ISOLATES OF *FUSARIUM SUBGLUTINANS*. A. Moretti[1], G. Mulé[1], A. Ritieni[2], M. Láday[3], G.P. Munkvold[4] and A. Logrieco[1]. *[1]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [2]Dipartiemento di Scienze degli Alimenti, Università di Napoli "Federico II", Via Università 100, 80055 Portici, NA, Italy. [3]Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary. [4]Department of Plant Pathology, Iowa State University, Ames, IA, 50011 USA. E-mail: antonio.moretti@ispa.cnr.it*

Fusarium subglutinans is a maize ear rot pathogen and producer of various mycotoxins including beauvericin (BEA). This species has recently been split into two major phylogenetic groups. A worldwide collection of 159 isolates of the fungus, mostly from Europe and Iowa, USA, originating from maize, was subjected to phylogenetic and RFLP analysis and to chemical analysis for BEA production. Of the 64 isolates belonging to Group 1, 48 (75%) produced from 10 to 532 µg/g of BEA, whereas none of the 95 Group 2 isolates synthesized detectable amounts of the mycotoxin. The association between RFLP group and BEA production is consistent with the existence of two reproductively isolated subgroups within *F. subglutinans* and indicates that the toxicological risk of isolates of *F. subglutinans* may vary between the groups. Finally, a higher number of isolates from different geographical areas is needed in order to better evaluate the potential toxicological exposure of maize worldwide.

MYCOTOXIN PRODUCTION AND MOLECULAR ANALYSIS OF *FUSARIUM FUJIKUROI* ISOLATED FROM BAKANAE DISEASED RICE PLANTS IN NORTHERN ITALY. A. Moretti[1], A.M. Picco[2], M. Rodolfi[2], S. Somma[1], A. Ritieni[3] and R. Ferracane[3]. *[1]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/0, 70126 Bari, Italy. [2]Dipartimento di Ecologia del Territorio e Ambienti Terrestri, Sezione di Micologia, Univer-*

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Fusarium fujikuroi (sexual stage *Gibberella fujikuroi*) is a fungal pathogen causing bakanae disease of rice worldwide. Bakanae has emerged in recent years as a major disease of rice in Northern Italy. In 2004 and 2005 we isolated 142 strains of *Fusarium* from stems and roots of rice plants showing the most typical and well-known symptom of bakanae, abnormal stem growth. Despite the possibility that other *Fusarium* species can be involved in the disease, we identified only *F. fujikuroi* among the strains studied, with few exceptions. The identification of the strains was confirmed by testing their sexual fertility, by using DNA analysis, in particular sequencing ß-tubulin and elongation factor loci, and comparison with AFLP analyses, which were performed in order to analyze the genetic diversity of this set of *F. fujikuroi* strains. Moreover, the strains were subjected to chemical analysis for production of the mycotoxins beauvericin, enniatins, fumonisin B_1 and B_2, and fusaproliferin. The data showed that many strains could produce high levels of enniatins (50% of strains up to 2000 µg/g) and beauvericin (70% of strains up to 5000 µg/g) *in vitro* culture of rice, whereas the more dangerous fumonisins, which are well known carcinogenic compounds, were produced at low levels only by around 30% of strains (up to 120 µg/g total fumonisins). Finally a characterization of FUM genes was carried out for the *F. fujikuroi* strains in order to screen the distribution of the FUM genes among the fumonisin-producing and non-producing strains.

FVVE1 REGULATES FUMONISIN PRODUCTION AND PATHOGENICITY IN _FUSARIUM VERTICILLIOIDES_. **K. Myung[1], S. Li[1], N. Zitomer[2], H.K. Abbas[3], A.H. Glenn[2] and A.M. Calvo[1].** *[1]Department of Biological Sciences, Northern Illinois University, 1425 W. Lincoln Hwy., DeKalb, Illinois 60115, USA. [2]Toxicology and Mycotoxin Research Unit, USDA-ARS, Russell Research Center, Athens, Georgia 30605, USA. [3]Crop Genetics and Production Research Unit, USDA-ARS, Stoneville, Mississippi 38766, USA. E-mail: amcalvo@niu.edu*

The *veA* homologous genes are essential for biosynthesis of sterigmatocystin in *Aspergillus nidulans* and aflatoxin production in *A. parasiticus* and *A. flavus*. Whether *veA* homologs have a role in regulating secondary metabolism in other fungal genera is unknown. In this study, we examined the role of the *veA* homologous gene *FvVE1* on production of fumonisin toxins in the important plant pathogen *F. verticillioides*. Our studies indicate that deletion of *FvVE1* suppresses fumonisin production on corn (maize) and rice medium. Furthermore, corn plants grown from seeds inoculated with *FvVE1* deletion mutants did not show disease symptoms while plants grown from seeds inoculated with the *F. verticillioides* wild type and complementation strains clearly showed disease symptoms under the same experimental conditions. In this latter case, the presence of lesions coincided with accumulation of fumonsins in the plant tissues, and only these plant tissues had elevated levels of sphingoid bases, indicating disruption of sphingolipid metabolism. The effects of *FvVE1* deletion on toxin production and plant disease were found to be the same in two separate mating types. Our results strongly suggest that *FvVE1* is necessary for fumonisin biosynthesis and pathogenicity by *F. verticillioides*. The conservation of *veA* homologs among ascomycetes suggests that *veA* could play a pivotal role in regulating secondary metabolism and pathogenicity in other fungi. We propose *veA* as a potential target for implementation of a control strategy to prevent the devastating health and economic effects of plant pathogenic fungi.

GEOGRAPHIC DISTRIBUTION OF _FUSARIUM_ spp. AND PRODUCTION OF FUMONISINS IN MAIZE OF SUBSISTENCE FARMERS IN SOUTH AFRICA. **E. Ncube[1,3], B.C Flett[1], C. Waalwijk[2] and A. Viljoen[3].** *[1]ARC-GCI, Private Bag X1251, Potchefstroom 2520, South Africa. [2]Plant Research International B.V., P.O. Box 16, 6700 AA Wageningen, The Netherlands. [3]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. E-mail: ncubee@arc.agric.za*

Fusarium spp. produce mycotoxins in maize that are carcinogenic to humans and animals, called fumonisins. These fumonisins are most damaging in rural agricultural systems where people and animals often rely on all food and feed available on their lands. To determine the distribution and importance of fumonisin-infected maize in the rural areas of South Africa, maize samples were collected in four provinces in South Africa during 2005/06 and 2006/07. Mycotoxin levels were quantified using ELISA, and the incidence and quantity of *Fusarium* spp. in maize was determined by plate counts and real-time PCR, respectively. A questionnaire was further compiled to investigate agricultural practices in rural areas that may influence mycotoxin contamination of maize. *F. verticillioides* was the most common *Fusarium* species. found in maize followed by *F. subglutinans* and *F. proliferatum*. KwaZulu-Natal, Eastern Cape and Limpopo provinces were most affected by fumonisins. Fumonisin levels in maize samples ranged from 0-21.8 ppm while real-time PCR results ranged from 0-1965.5 pg *Fusarium* DNA/mg tissue. Regression analyses showed a positive correlation between fumonisin concentration and fumonisin-producing *Fusarium* species when determined by real-time PCR (r^2=0.866). Mycotoxin levels measured were in excess of the maximum levels set by the European Union and the FDA in the USA. Regulations on maximum permitted levels of fumonisin in food and feed are currently unavailable in South Africa. The high incidence of mycotoxin contamination in subsistence farming systems indicates the need for awareness programmes and further research.

FUSARIUM INFECTION AND MYCOTOXINS IN FINNISH CEREALS IN 2005-2006. **P. Parikka[1] , S. Rämö[1] and V. Hietaniemi[2].** *[1]MTT Agrifood Research Finland, Plant Production Research, 31600 Jokioinen, Finland. [2]MTT Agrifood Research Finland, Laboratories, 31600 Jokioinen, Finland. E-mail: paivi.parikka@mtt.fi*

Fusarium infection and mycotoxins were investigated on dried cereal grain in 2005-2006. Altogether, 388 samples were collected from farms in different regions in Finland. The main species investigated was oat, followed by barley and spring wheat. Winter wheat and rye were less sampled. In 2005, the weather was more favourable to *Fusarium* infection, and the deoxynivalenol (DON)-producing species *Fusarium culmorum* and *F. graminearum* were common on oat and barley, less common on spring wheat. The T2/HT-2-producing species *F. sporotrichioides* and *F. langsethiae* were most often detected on oat cultivars, less on barley. In the dry conditions of 2006, DON-producing species were not so common as in 2005, but T2/HT-2-producing species and *F. poae* were more abundant. Especially *F. langsethiae* was quite often detected on certain oat cultivars, also in samples derived from northern locations. The fungus was detected on all cereal species investigated. This species seems to be the most important T2/HT-2 producer on Finnish cereals. Particularly these toxins are being analysed on oats. The highest detected DON contents occurred on oats. In 2005, some samples of barley and spring wheat also had higher DON contents, but in 2006 the contents analysed did not exceed the limits of EU regulations. The ni-

valenol contents were normally below 200 µg/kg, but in 2005 in some oat samples the contents were significantly higher. Zearalenone was not often detected on cereals. The samples investigated did not contain DAS, F-X or 15-AcDON, and 3-AcDON was only detected in 2005 when the DON contents were high.

REPORTS OF FUSARIAL-TOXIN POISONING IN INDIA. B.N. Reddy and C.R. Raghavender. *Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad 500007, India. E-mail: reddybn1@yahoo.com*

Fusarial toxins are metabolites produced by certain species of toxigenic *Fusarium*. This group of mycotoxins includes trichothecenes (such as T-2 toxin, deoxynivalenol and nivalenol), zearalenone and fumonisins. Fusarial toxins are gaining increasing importance due to their deleterious effects on human and animal health, particularly in India where diets are highly prone to these toxins due to poor harvesting practices coupled with improper storage conditions resulting in chronic health risks. This paper reviews the fusarial toxin outbreaks in India due to ingestion of mycotoxin-contaminated food and feed. Trichothecenes have involved in an acute human mycotoxicosis known as alimentary toxic aleukia (ATA) in India during 1987 and attributed to the consumption of mouldy wheat affecting 97 members. T-2 toxin has been implicated in several animal diseases including an outbreak of acute human mycotoxicoses. Information on trichothecene and zearalenone poisoning in animals is scanty in India. Many acute disease outbreaks due to exposure to DON have also been reported. An outbreak of acute food-borne disease caused by fumonisin has been reported in south India during 1995 affecting 1,424 people due to consumption of contaminated sorghum and maize. These outbreaks continue to be the problems of significant health in India because of poor purchasing ability that compels people to consume cheap and contaminated food. Thus, monitoring human population groups for diseases attributable to mycotoxins, coupled with implementing appropriate prevention and control measures, including decontamination and detoxification, would ensure a wholesome food supply free from mycotoxins.

PATHOGEN TOXINS OF BAYOUD DISEASE ON DATE PALM: *IN VITRO* SELECTION AND BIOLOGICAL AND SPECIFICITY ACTIVITIES. My. H. Sedra[1], H.B. Lazrek[2], H. Amraoui[3] and S. Nour[1]. *[1]Arab Organization for Agricultural Development (AOAD)/ Institut National de Recherche Agronomique (INRA), Laboratory of Phytopathology, Genetics and Integrated control, Regional Centre BP.533, Marrakech, Morocco. [2]Department of Biology, Faculty of Sciences and Techniques, University Cadi Ayyad, BP 549, Marrakech, Morocco. [3]Department of Chemistry. Faculty of Sciences Semlalia, University Cadi Ayyad, B.P S 15, Marrakech, Morocco. E-mail: sedramh@hotmail.com and mhsedra@yahoo.fr*

Bayoud, caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* is the most serious disease of the date palm (*Phoenix dactylifera L.*) in North Africa. The selection for resistant date palm varieties is the privileged way to control the disease. The use of pathogen toxin *in vitro* selection could be the good approach for rapid method of screening. We produced different fractions of toxins: from 15 l of Czapek medium, 1310 mg of 3 toxic fractions (FI, FII and FII) have been produced (at the rate of 87,3 mg/l) of which 488 mg were of FII fraction. In a second trial we compared the *in vitro* toxic effect of several types of toxic substances (with different concentrations), notably the fusaric acid (FA) commercial product), the culture filtrate and the FII toxin extracted from of this filtrate, on the embryos from seed descended from two date palm varieties: susceptible (Jeehel (JHL) and resistant (Black Bousthammee (BSTN)). Both compared toxic substances affect the embryos. The symptoms were: rootlets browning, a delay of the gemmule apparition or the inhibition of seedling growth. The treatment effect depended on the variety and the concentrations tested. The filtrate was the most toxic follow-up of the FII statically then the FA. This could be explained by the fact that the filtrate contains the FII fraction and the FA. In a third trial we evaluate the toxicity of both fractions extracted on detached leaflets of different plant species. On detached leaves, the symptoms were: coiling and drying up that conduced to their death. The pathogen toxins affected all tested species. The FII fraction was the most toxic globally.

THE VIRULENCE OF *FUSARIUM* ISOLATES AND THEIR ABILITY OF MYCOTOXIN PRODUCTION. L. Slezakova, T. Sumíková and F. Kocourek. *Crop Research Institute, Drnovska 507, 16106 Praha 6, Ruzyne, Czech Republic. E-mail: Slezakova@seznam.cz*

During two years the virulence of the most frequent *Fusarium* species on maize (*Fusarium graminearum, F. verticillioides* and *F. subglutinans*) and their ability to produce mycotoxins were evaluated. Corn cobs were infected by single isolates of these species and after harvest the occurrence of selected mycotoxins (DON, 3, 15 - ADONs, NIV, HT-2 toxin, T-2 toxin, FUS-X and ZEA) in the samples were assessed by HPLC-MS. DON concentrations in the range of 12,14 - 107 513,91 µg/kg were analysed from all infected corn cobs. The highest DON concentrations in all grain samples inoculated by *F. graminearum* were presented. Similarly the mycotoxin NIV in concentration range of 8,88 - 2594,44 µg/kg in all samples were analysed. The other selected mycotoxins ADONs, FUS-X and ZEA were also found. Only the mycotoxins HT-2 and T-2 toxins were not found in any samples. For characterization of mycotoxin chemotype profile of these isolates PCR assays were used. All *F. graminearum* were detected as deoxynivalenol producers and all *F. verticillioides* and *F. subglutinans* as fumonisin producers.

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THE STRUCTURAL ANALYSIS OF ENNIATIN H, I AND MK1688 AND BEAUVERICIN BY LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY (LC-MS/MS) AND THEIR PRODUCTION BY *FUSARIUM OXYSPORUM* KFCC 11363P. H.H. Song, H.S. Lee, J.H. Jeong and C. Lee. *Department of Food Science and Technology, BET Research Institute, Chung-Ang University, Ansung, 456-756, Korea. E-mail: chanlee@cau.ac.kr*

The molecular structures of enniatins H, I, and MK1688 and beauvericin were investigated by liquid chromatography-tandem mass spectrometry (LC-MS/MS). They were fragmented by loss of -CO after opening of the cyclic molecule to carbonyl carbon, and cleavage of the peptide and ester bonds in the molecular structure was elucidated by LC-MS/MS spectrometry. *Fusarium oxysporum* KFCC 11363P was tested for its ability to produce beauvericin and enniatins H, I, and MK1688 on five cereal substrates: rice, barley, maize, wheat, and Indian millet kernels. Furtheremore, optimal conditions for the production of tested mycotoxins by the *Fusarium* isolate were tested on maize at four tem-

peratures (15, 20, 25, and 30°C) and for three moisture contents (10, 20, and 40%). Large amounts of beauvericin and enniatin H were present in maize cultures at 25°C (232.4 and 196.4 μg g^{-1}, respectively). Enniatins I and MK1688 were maximally formed at 20°C (221.5 and 180.2 μg g^{-1}, respectively). The optimal moisture contents for the production of enniatins H (196.4 μg g^{-1}) and MK1688 (165.6 μg g^{-1}), were 40%.

DIVERSITY IN BEAUVERICIN AND ENNIATINS H, I, AND MK1688 BY *FUSARIUM* SPECIES ISOLATED FROM POTATO. H.H. Song, H.S. Lee, H. Li and C. Lee. *Department of Food Science and Technology, BET Research Institute, Chung-Ang University, Ansung, 456-756, Korea. E-mail: chanlee@cau.ac.kr*

Beauvericins and enniatins are cyclohexadepsipeptide mycotoxins that exhibit phytotoxicity and insecticidal activities. In the present study, the production of beauvericin and newly found enniatins (H, I, and MK1688) was characterized in 28 *Fusarium* strains isolated from potato samples in Korea. The predominant *Fusarium* species in potato was *F. oxysporum* (53.6%). Fifteen strains of *F. oxysporum* and two strains of other *Fusarium* species produced beauvericin (at concentrations from 3.1 to 743.2 μg/g) in culture on rice. Enniatins H and I were produced by 3 and 11 strains at concentrations from 33.1 to 781.3 μg/g and from 6.5 to 730.3 μg/g, respectively. Five isolates produced enniatin MK1688 at concentrations from 4.6 to 432.6 μg/g. In particular, one isolate (No. 1501) identified as *F. oxysporum* and two other *Fusarium* strains (Nos. 804 and 910) produced all of the tested toxins. These results indicate that enniatins H, I, and MK1688 and beauvericin are produced by *Fusarium* isolates occurring on potato. We do not know if the toxins can accumulate in the environment since it was not demonstrated.

***FUSARIUM AVENACEUM* AND ITS METABOLITES IN APPLES SHOWING WET CORE ROT SYMPTOMS. J.L. Sørensen[1], H.-J. Schroers[2], U. Thrane[1], K.F. Nielsen[1], M. Zerjav[2], A. Munda[2] and J. Frank[2].** *[1]Technical University of Denmark, Department of Systems Biology, Søltofts Plads 221, 2800 Kgs. Lyngby, Denmark. [2]Agricultural Institute of Slovenia, Hacquetova 17, 1001 Ljubljana, Slovenia. E-mail: jls@biocentrum.dtu.dk*

Occurrence and causal agents of wet apple core rots (wACR) were studied in Slovenian orchards in 2004-2006. White, rose or reddish mycelium is first developing in the core of the apples and a brown, wet rot expends destructively into the surrounding flesh. In a study of the full yield of 21 Gloster trees, ca 10% of the apples developed a wACR. *Fusarium avenaceum* could almost always be isolated from the rotten apples and was the primary cause of wACR also in other cultivars such as Golden Delicious, Fuji and Jona Gold. However, infection rate of Golden Delicious apples was less than 2%. *F. avenaceum* strains from apples formed, among others, the metabolites antibiotic Y, two chlamydosporols, aurofusarin, rubrofusarin and several enniatines, identified qualitatively in HPLC DAD/MS analysis, on artificial agar media. Occurrence of some of these important metabolites was also studied in apple rots. These studies focused mainly on moniliformin, but antibiotic Y and aurofusarin were also included. Moniliformin was found in levels up to 9000 ppb in naturally infected samples. Artificial inoculations of rot-free apples with representative strains of *F. avenaceum* indicated that moniliformin is formed after 3-7 days in small amounts and can reach levels of 2000-4500 ppb within 3 weeks. Because apples with wACR can be difficult to identify and remove from the production processes, these results indicate that mycotoxins produced by *F. avenaceum* can be a threat to human health.

MYCOTOXIN PRODUCTION BY *FUSARIUM PROLIFERATUM* AND *F. VERTICILLIOIDES* ISOLATED FROM HOPS IN SERBIA. S. Stankovic[1], J. Levic[1], T. Petrovic[1] and V. Krnjaja[2]. *[1]Laboratory of Phytopathology, Maize Research Institute "Zemun Polje", Slobodana Bajica 1, Belgrade-Zemun, 11080, Republic of Serbia. [2]Institute of Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia. E-mail: sstojkov@mrizp.co.yu*

Hop (*Humulus lupulus* L.) is commercially important as an essential flavoring in beer and is cultivated on no more than 600 ha in Serbia, although industry requirements are much greater. Fungi from the genus *Fusarium* can cause wilting, chlorosis and canker of hops in most hop-growing countries. Among them, *F. sambucinum* is the most frequent species. *F. proliferatum* has been rarely described as a parasite of hops, and for *F. verticillioides* no data is available. The objectives of this study were to investigate the toxicological profile of the *F. proliferatum* and *F. verticillioides* strains isolated from diseased hop plants, to study their possible fertility and assign them to a specific mating population. For fertility test, all strains were crossed twice to standard tester strains of the MPs A through G (Klittich and Leslie, *Genetics* **118**: 417-423, 1988). Fumonisin B_1, beauvericin and fusaproliferin extractions and analyses were performed by HPTLC according to the procedures described by Logrieco *et al*. (*J. Agric. Food Chem.* **41**: 2149-2152, 1993; *Appl. Environ. Microbiol.* **62**: 3378-3384, 1996), respectively. Mycotoxin production of strains, tested for pathogenicity, was evaluated and showed that all isolates of *F. proliferatum* and *F. verticillioides* produced 250 to 3000 μg g^{-1} fumonisin B_1; five out of six isolates of *F. proliferatum* produced 400 to 500 μg g^{-1} beauvericin; three strains of *F. proliferatum* produced 400 to 450 μg g^{-1} fusaproliferin and all isolates of *F. verticillioides* produced up to 400 μg g^{-1} fusaproliferin. All tested strains of *F. proliferatum* were MATD-2 and MATA-1 for *F. verticillioides*.

***FUSARIUM* SPECIES, DEOXYNIVALENOL (DON), 15-ADON AND 3-ADON IN NATURALLY INFECTED AND INOCULATED WHEAT IN ONTARIO, CANADA. L. Tamburic-Ilincic[1], D. Gaba[2], T. Nowicki[2] and A. Schaafsma[1].** *[1]Ridgetown Campus, University of Guelph, Ridgetown, Ontario, Canada. [2]Grain Research Laboratory, Canadian Grain Commission, Winnipeg,, Manitoba, Canada. E-mail: ltamburi@ridgetownc.uoguelph.ca*

Fusarium graminearum (Schwabe) causes Fusarium head blight (FHB), an important wheat disease. Deoxynivalenol (DON) is the most important mycotoxin produced by *F. graminearum*; 15-ADON and 3-ADON analogs may also be produced. In the first study we identified *Fusarium* species and measured level of DON from commercial wheat fields in Ontario from 2004 to 2007 and level of 15-ADON and 3-ADON from wheat fields in 2007. The top three *Fusarium* species identified were *F. graminearum, F. sporotrichioides* and *F. poae.* The highest level of DON was detected in 2004, ranging 0.8 to 4.9 ppm. No detectable levels of 15-ADON and 3-ADON were produced in wheat grain in 2007 In the second study, three wheat cultivars were spray-inoculated in greenhouse with three 15-ADON and three 3-ADON isolates of *F. graminearum* from Ontario. Fusarium damaged kernel (FDK) content of the harvested grain was established visually and *Fusarium* mycotoxins were measured by GC-MS. The highest level of 15-ADON in Roblin, Winfield and Hobson cultivars, inoculated with 15-ADON isolates was 1.3

ppm, 0.2 ppm and 0.2 ppm, respectively. After inoculation with 15-ADON isolates, percent of FDK in Roblin, Winfield and Hobson, on average was 50.3%, 26.3% and 36.0%, respectively, while DON level ranged from 1.8 to 35.0 ppm. 3-ADON was detected (0.2 ppm) only in the most susceptible cultivar (Roblin) inoculated with one 3-ADON isolate; 13 ppm of DON was detected in the same sample. Percent of FDK in Roblin, Winfield and Hobson, after inoculation with 3-ADON isolates, on average was 21.9%, 8.9% and 19.9%.

MYCOTOXIC METABOLITES PRODUCED BY *FUSARIUM* SPECIES ASSOCIATED WITH FUSARIUM HEAD BLIGHT AND FEED REFUSAL DISORDERS IN WESTERN AUSTRALIA. D. Tan[1], E. Ghisalberti[2], G. Flematti[2], M.J. Barbetti[1,4] and K. Sivasithamparam[3]. *[1]School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia. [2]School of Biomedical, Biomolecular and Chemical Sciences, Faculty of Life and Physical Sciences, The University of Western Australia, Crawley, WA 6009, Australia. [3]School of Earth and Geographical Sciences, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia. [4]Department of Agriculture and Food Western Australia, South Perth, WA 6151, Australia. E-mail: tand12@student.uwa.edu.au*

Fusarium Head Blight (FHB) in wheat is one of the world's most destructive plant diseases, decreasing both grain yield and quality. In 2004, FHB was detected on quality assurance samples of wheat grain and on summer cereal crop residues in Western Australia. There have been earlier reports on FHB outbreaks from eastern Australia, Canada, China, southern and eastern Europe, Japan, South America, and the USA. Accounts of the occurrence of *Fusarium* as plant pathogens are widespread and worldwide, and various *Fusarium* spp. have been found to attack a wide range of plants, including pasture species, cereals, maize, cotton, banana, and tomato. Sheep grazing in six different locations in Western Australia were recently reported to partially or completely refuse to ingest annual *Medicago* pods, which were later found to be contaminated with a number of different *Fusarium* species known to produce deoxynivalenol (causes vomiting and feed refusal in animals) and/or diacetoxyscirpenol (causes reduced feed intake in animals) as part of their array of toxigenic secondary metabolites. Similar incidents have also been reported in South Africa and Central United States. This study aims to isolate and characterize the secondary metabolites produced by Western Australian *Fusarium* species, associated with cereal and pasture species, which have caused *Fusarium* head blight and/or feed refusal disorders in sheep, respectively.

EARLY EVALUATION OF MYCOTOXIN CONTAMINATION RISK IN MAIZE. E. Torelli[1], G. Bianchi[2], F. Saccardo[2], R. Locci[1] and G. Firrao[1]. *[1]Dipartimento di Biologia e Protezione delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. [2]ERSA, Agenzia Regionale per lo Sviluppo Rurale, Via Sabbatini 5, 33050 Pozzuolo del Friuli, Udine, Italy. E-mail: torelli@uniud.it*

In north-east Italy the occurrence of mycotoxins on maize infected before harvest is of great concern for food and feed safety. To prevent the introduction into the food chain of contaminated lots of grain, there is an urgent need for rapid methods for the early assessment of contamination, since the extraction and analysis of samples is a time-consuming process that is not suitable for routine analysis at the time of delivery of the grain to drying and storage services. Here, we report the development and the evaluation of proximal imaging analysis with near infrared illumination for the early assessment of contamination risk: this work investigates the potential of proximal imaging analysis for rapid classification of grains according to their contamination levels. The prediction data were correlated with the content of different toxins, fumonisin, aflatoxin, ochratoxin, deoxinivalenol, zearalenone, in about 300 field collected maize samples as determined by ELISA and HPLC. Previous studies showed that an effective model can be developed that predicts the amounts of FB1 from location/weather data and the amount of toxigenic fungal spores. Therefore agronomic (hybrid, seed date, harvest date, water content at harvest, irrigation, and pest management) and environmental data were also integrated in the aim of developing risk assessment models and protocols for the Friuli Venezia Giulia region.

***FUSARIUM* SPECIES IN WINTER WHEAT IN LATVIA: DISTRIBUTION AND TOXIGENIC EFFECT. O. Treikale, I. Priekule, J. Pugacheva, L. Lazareva.** *Latvian Plant Protection Research Centre, Lielvardes street 36/38, 1006 Riga, Latvia. E-mail: olga.treikale@laapc.lv*

The aim of this study was to evaluate the distribution of species associated with Fusarium head blight in winter wheat in Latvia and affects on contamination of yield with mycotoxins. From ear samples representing several winter wheat varieties cultivated in various regions of Latvia under the different agricultural practices nine *Fusarium* species were identified. *F. culmorum, F. avenaceum* var. *herbarum*, *F. gibbosum, F. poae*, *F. oxysporum* var. *orthoceras, F. sambucinum*, *F. sporotrichoides, F. moniliforme* and *F. semitectum*. During the years of investigation four *Fusarium* species were prevalent: *F. poae, F. culmorum, F. gibbosum* and *F. avenaceum* var. *herbarum.* The contamination with mycotoxins (deoxinivalenol, zearalenone, T-2 toxin) was examined in harvested grain from 64 (2006) and 81 (2007) sites. The level of mycotoxins in grain samples was low. In 2006 DON was presented in 4.7% of samples (28-36 µg/kg), ZEN and T-2 toxin were not found. In 2007 DON was found in 27.2% of samples (1.0-615.0 µg/kg), ZEN in 2.5% of samples (1.7-9.3 µg/kg), T-2 toxin was detected in 3.7% of samples (2.5-64.0 µg/kg). Analysis of variance showed moderate dependency of contamination of yield with DON from severity of Fusarium Head Blight on ear ($r = 0.490$, *P*- level 0.02). In inoculated field trials over two years the effect of fungicides on development of Fusarium head blight and influence on reduction of contamination of yield with deoxynivalenol were determined. There was observed that DON content in harvested yield associated with incidence of *F. culmorum* in grain of winter wheat.

METABOLOMIC AND PHENOTYPIC ANALYSES OF *FUSARIUM GRAMINEARUM* WILD-TYPE AND MUTANT STRAINS. M. Urban[1], R. Lowe[1], W. Allwood[1], J. Ward[2], M. Beale[2] and K. Hammond-Kosack[1]. *[1]Department of Plant Pathology and Microbiology. [2]National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Herts, AL5 2JQ, UK. E-mail: kim.hammond-kosack@bbsrc.ac.uk*

The trichothecene mycotoxin producing Ascomycete fungus *Fusarium graminearum* causes ear blight disease of small grain cereals. Infections lower grain quality and safety, and are of increasing global concern. We analysed shifts in the fungal metabolome of two *F. graminearum* wild-type strains PH-1 and 16A and three virulence mutants affected in either catabolite repression, MAP

kinase cell signalling or mycotoxin production. Various *in vitro* growth conditions were used and the intracellular metabolome was investigated by global 1H-NMR and ESI-MS. The various *in vitro* and *in planta* phenotypes displayed by the *SNF1* gene deletion mutant (fg09897) will be presented. In addition, the metabolite changes in all three mutants will be correlated to the function of the affected gene product. The use of metabolomics to help phenotype fungal pathogenicity mutants is discussed.

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VARIANCE IN FUMONISIN QUANTIFICATION IN MAIZE SAMPLES IN SOUTH AFRICA USING THE ELISA VERATOX PROTOCOL. B.J. van Rensburg[1], B.C. Flett[1], N.W. Mc Laren[2] and A.H. Mc Donald[1]. *[1]ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa. [2]Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa. E-mail: BelindaJ@arc.agric.za*

Fumonisins produced by *Fusarium verticillioides* and *F. proliferatum* cause mycotoxicoses in horses, swine and rats and have been statistically associated with oesophageal cancer in humans. Accurate measurement of mycotoxins is essential for determining the safety of grain and their products for consumption. Four sources of variation were studied, namely sub-sample size, variation within a single maize sub-sample, number of replicates and the comparison of ELISA and HPLC results. Variation in fumonisin levels within a single maize sample was high using the 25 g sub-samples proposed in the Neogen Veratox protocols. A 250 g sub-sample significantly reduced variation in fumonisin levels of samples. An incremental increase in sample size also improved the number of positive samples recorded (R2=0.81). Increasing the number of replicates using the recommended sub-sample size (25 g) did not reduce variation except when the sample had high fumonisin levels. Improved accuracy was recorded when a 250 g sub-sample was used in conjunction with increased replicates. Data from laboratory analyses indicated that ELISA reactions (Agricultural Research Council – Grain Crops Institute) correlated significantly with HPLC results of the Medical Research Council (MRC). Concentrations determined using ELISA were consistently higher by 33% than those from the HPLC (MRC) results. Quantification technique, sample size, replicate number and laboratory where analyses are conducted, are important sources of variation for quantification of fumonisins.

ETHANOL CO-PRODUCTS CONTAMINATED WITH MYCOTOXINS: COSTS TO LIVESTOCK INDUSTRY. F. Wu[1] and G.P. Munkvold[2]. *[1]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA. [2]Department of Plant Pathology, Iowa State University, Ames, IA, USA. E-mail: few8@pitt.edu*

The rapidly expanding U.S. ethanol industry is generating a growing supply of co-products, mostly in the form of dried distillers' grains and solubles (DDGS) or wet distillers' grains (WDG). In the US, 90% of the co-products of maize-based ethanol are fed to livestock. An unintended consequence is that animals may be fed higher levels of mycotoxins, which are concentrated up to three times in DDGS compared to grain. Many animal species experience adverse multiple health effects from mycotoxin consumption; for example, swine exposed to fumonisin experience symptoms ranging from reduced weight gain to cardiovascular dysfunction to pulmonary edema. The model developed in this study estimates swine industry losses from weight gain reduction due to fumonisin in added DDGS. At current levels of DDGS adoption in U.S. swine feed, the expected cost from weight gain reduction is about $10 million ($2-$18 million) annually. If there is complete market penetration of DDGS in swine feed, and fumonisin is not controlled, losses may increase to about $150 million annually. This represents only losses attributable to one mycotoxin on one adverse outcome on one livestock species; the total loss due to mycotoxins in DDGS could reach the billions USD if additional mycotoxins and livestock species are considered. If ethanol producers implement mycotoxin surveillance, the losses are shifted among multiple stakeholders. Solutions to this problem include methods to reduce mycotoxin contamination in both pre-harvest and post-harvest maize.

TRICHOTHECENE CHEMOTYPE COMPOSITION OF *FUSARIUM GRAMINEARUM* AND RELATED SPECIES IN FINLAND AND RUSSIA. T. Yli-Mattila[1], K. O'Donnell[2], T. Ward[2] and T. Gagkaeva[3]. *[1]Laboratory. of Plant Physiology. and Molecular Biology, Department of Biology, University of Turku, 20014 Turku, Finland. [2]Microbial Genomics Research Unit, USDA-ARS, Peoria, USA. [3]Laboratory of Mycology and Phytopathology, All-Russian Institute of Plant Protection (VIZR), St. Petersburg, Russia. E-mail: tymat@utu.fi*

Fusarium graminearum and type B trichothecene producers can be divided into three chemotypes. Analysis of 290 single-spore isolates of *F. graminearum* and related *Fusarium* species revealed that all *F. graminearum* isolates from Finland (15) and western Russia (26) possessed the 3ADON chemotype, while >90% of the isolates from southern Russia (45) possessed the 15ADON chemotype. In other parts of Russia and northern China both chemotypes were present. The only *F. graminearum* isolate with the NIV chemotype was from Germany. All 27 *F. culmorum* isolates (Finland and Russia) possessed the 3ADON chemotype, whereas all six isolates of *F. cerealis* possessed the NIV chemotype. These results are in accordance with the results of other chemotype-specific primers and mycotoxin analyses of pure cultures. In Finland there were no differences in the *F. graminearum* chemotype composition between the years 1986-93 and 2001-2004, while in the Far East (90 isolates) the 3ADON chemotype frequency increased between the years 1998-2006. This apparent shift in trichothecene chemotype frequency is similar to recently observed shifts in *Fusarium* head blight pathogen composition within North America. Two Russian *F. graminearum* isolates produced a positive signal with a 3ADON and 15ADON multilocus genotyping assay probe from opposite ends of the trichothecene gene cluster, suggesting that it may reflect recombination between isolates with these two chemotypes. Twelve isolates from Far East and Siberia produced unusually low positive signals for the *F. graminearum* probes. These isolates likely harbor previously unrecognized variation at the probe sites and will be sequenced.