



**MOLECULAR CHARACTERIZATION OF *FUSARIUM* DAMPING-OFF IN FOREST NURSERIES IN SPAIN.** F.M. Alves-Santos, J.A. Pajares and J.J. Diez. *Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Escuela Técnica Superior de Ingenierías Agrarias de Palencia, Avenida de Madrid 57, 34071 Palencia, Spain. E-mail: jdcasero@pvs.uva.es*

Several conifer tree species are known to be affected by damping-off caused by different fungal and fungal-like species. In this complex the pathogenic fungi *Fusarium oxysporum* and *F. verticillioides* have been isolated frequently. Ten *F. oxysporum* isolates and 13 *F. verticillioides* isolates recovered from diseased trees were analyzed by random primed amplification. Based on intensity and reproducibility of polymorphic amplified fragments, a total of 22 RAPD markers were recorded, ranging from two to seven markers for primer. Two of these markers (OPC2.1 and OPC6.1) were amplified from Fo2 isolate (*F. oxysporum*). Other markers as OPC6.3 and OPC18.5 were present in most of *F. oxysporum* isolates and absent in *F. verticillioides*. None of RAPD markers was present in *F. verticillioides* and absent in *F. oxysporum*. RAPD data was analysed with phylogenetics software and the resulting phylograms allow us to separate a *F. oxysporum* group and a larger group where both *Fusarium* species were present. On this major group a *F. verticillioides* subgroup could be established. When including the ITS haplotypes on the resulting tree some relations have been observed. The large group showed mostly haplotype B+ (larger fragment with *Pst*I restriction site) whereas the small group showed all ITS haplotypes and included the nonpathogenic isolates. It was surprising that no species correlation have been registered. Our results suggest that RAPD analysis is useful for measuring degrees of genetic relationship among populations of closely related fungal pathogens but it would be convenient not to use RAPD markers as single diagnostics method.

**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *FUSARIUM LATERITIUM* FROM HAZELNUT IN ITALY.** A. Belisario, S. Vitale and A. Santori. *CRA Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: alessandra.belisario@entecra.it*

*Fusarium lateritium* has been isolated from numerous woody trees and shrubs where it may cause wilt, tip or branch dieback, or cankers. In Italy this fungus was recently described as the main agent of gray necrosis (GN) on hazelnut fruit and the cause of twig cankers. A collection of *F. lateritium* isolates obtained from different geographical origins and hosts were identified and characterized by cultural and morphological features. Inter-simple sequence repeat (ISSR) markers were used to investigate genetic diversity among 50 isolates of *F. lateritium*. Fifteen among 26 primers tested were chosen for their reproducibility and high polymorphism. Intraspecific variability among isolates was revealed. The association between *F. lateritium* isolate profiles with colony morphology, host, and geographic origin is discussed.

**STRATEGIES TO REDUCE *FUSARIUM* AND MYCOTOXIN CONTAMINATION IN NORWEGIAN CEREALS.** G. Brodal, O. Elen, I.S. Hofgaard, H.U. Aamot and S.S. Klemsdal. *Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Hogskoleveien 7, 1432 Aas, Norway. E-mail: guro.brodal@bioforsk.no*

A strategy for identification of cereal grain lots with unacceptable mycotoxin content is under development: (i) prediction

models will estimate the risk for *Fusarium* infection and/or mycotoxin development in cereal fields based on information about agronomic and climatic conditions; (ii) grain from high-risk fields will be analysed by a rapid testing method before the grain lots arrive at the mill; (iii) samples from highly contaminated lots will undergo chemical analysis. During 2006 and 2007, approximately 300 samples of naturally infected grains of oats and spring wheat, have been collected from farms in the main cereal growing areas. Information about cultivation practice (tillage, previous crop), use of pesticides, soil conditions, sowing/heading/harvesting dates, and cereal species/cultivars has been registered for each grain sample. The samples are tested for *Fusarium* by real time PCR and analysed chemically for mycotoxin content, and the correlation with agronomic and climatic conditions is used to establish prediction models. Several assays (ELISA, dipstick, qPCR, microarray) are tested for their ability to assess the *Fusarium* and/or mycotoxin content in grains, and the results compared with chemical analyses, to find a rapid and suitable method for screening of a large number of samples. In addition to DON, ZON, T-2, HT-2 and NIV, also moniliformin and enniatins are included in the analyses. Samples from naturally infected fields, experimental fields, and materials from inoculation experiments in greenhouse, are included in the method studies.

**DEVELOPMENT OF A SCAR MARKER FOR THE MOLECULAR IDENTIFICATION OF THE FUNGUS *FUSARIUM SEMITECTUM*.** M. Dal Prà<sup>1</sup>, D. Rovito<sup>1</sup>, S. Tonti<sup>1</sup>, A. Prodi<sup>2</sup>, D. Pancaldi<sup>3</sup> and I. Alberti<sup>1</sup>. <sup>1</sup>Ente Nazionale delle Sementi Elette, Via Ca' Nova Zampieri 37, 37057 San Giovanni Lupatoto, Verona, Italy. <sup>2</sup>Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 46, 40127 Bologna, Italy. <sup>3</sup>Dipartimento di Protezione e Valorizzazione Agro-Alimentare, Università degli Studi, Viale Fanin 46, 40127 Bologna, Italy. E-mail: sezione-verona@ense.it

*F. semitectum* is a filamentous fungus showing remarkable characteristics, such as the adaptability to a great range of hosts and environments and the unique metabolites production pattern. Its importance is proved by the increasing number of scientific papers published in the last years regarding this species. *F. semitectum* infections were reported in cotton, banana, alfalfa, melon, soybean. This fungus was also isolated from rice, but its pathogenicity has still to be proven. *F. semitectum* can produce fibrinolytic enzymes, mycotoxins (type A trichotecenes, zearalenone), antibiotics (equisetin, epi-equisetin) and a broad range of metabolites with antifungal (fusapyrone, deoxyfusapyrone) or zootoxic (beauvericin) activity. In the present work we set up a new laboratory protocol for the molecular identification of this fungus. RAPD (Random Amplified Polymorphic DNA) technique was used to identify a species-specific amplification product. RAPD was combined with a fast DNA extraction method that generated reproducible band patterns. The selected fragment was cloned, sequenced and a primer pair (Fs1, Fs2) was developed to specifically detect *F. semitectum* using conventional PCR. The new SCAR marker was used to perform a mycological screening on rice, a species of great economical importance in Northern Italy.

**DIAGNOSIS OF *FUSARIUM OXYSPORUM* f. sp. *MELONIS* IN SOILS BY USING BAIT PLANTS.** M. de Cara<sup>1</sup>, D. Palmero<sup>2</sup>, V. López-Espinosa<sup>1</sup>, M. Santos<sup>1</sup> and J.C. Tello<sup>1</sup>. <sup>1</sup>Departamento de Producción Vegetal, Universidad de Almería, Carretera Sacramento s/n, 04120 Almería, Spain. <sup>2</sup>Universidad Politécnica de Madrid,



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We have developed a bait-plant method that consists in diluting the soil sample with sterile vermiculite at 1:6 (w:w) and adding it to a 1 L plastic glass, where six seeds of a susceptible muskmelon were sown. Then, glasses are moved into a climatic chamber (23 and 29°C, 60-99 RH, 16 h 12x103 lux/day fluorescent radiation). Between three to four weeks after sowing, plants start to show *Fusarium* wilt and necrotic xylem, if the soil was contained by *F. oxysporum* f. sp. *melonis*. Sections of stems can be plated on PDA and 48 h later the pathogen can be observed merging from the xylem. This bait-plant method and Warcup's classical method were used to analyse 72 soils from 24 farms with a known history of sudden wilt of melon, 4 or them from Mexico, 14 from Guatemala and 6 from Honduras. Six replicates were used per soil. *F. oxysporum* f. sp. *melonis* was detected in all Mexican soils by both methods, being inoculated 35 isolates obtained by Warcup's technique, and resulting 27 pathogenic to melon (67.6% of isolates). However, *F. oxysporum* f. sp. *melonis* was not detected in Guatemala and Honduras. One hundred *F. oxysporum* Warcup's isolates from Honduras and Guatemala were inoculated and did not show pathogenicity. Likewise, bait plants did not trap *F. oxysporum* f. sp. *melonis* from Guatemala and Honduras soils.

**EARLY DETECTION OF TOXIGENIC FUNGI ON MAIZE KERNELS BY SPECTRAL IMAGING ANALYSIS.** A. Del Fiore<sup>1</sup>, S. Serranti<sup>2</sup>, A. Ricelli<sup>3</sup>, M. Reverberi<sup>4</sup>, A.A. Fabbri<sup>4</sup>, A. Bonifazi<sup>2</sup> and C. Fanelli<sup>4</sup>. <sup>1</sup>Dipartimento Biotecnologie, Agroindustria e Protezione della Salute - ENEA, C.R. Casaccia, Via Anguillarese 301, 00123 Santa Maria di Galeria Roma, Italy. <sup>2</sup>Dipartimento di Ingegneria Chimica dei Materiali, delle Materie Prime e Metallurgia Università "La Sapienza", Via Eudossiana 18, 00184 Roma, Italy. <sup>3</sup>Istituto di Chimica Biologica-CNR, Piazzale Aldo Moro 5, 00185 Roma, Italy. <sup>4</sup>Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. E-mail: antonella.delfiore@casaccia.enea.it

Maize (*Zea mays* L.) is an important cereal, used for food and for animal feed. Several toxigenic fungi can infect maize in the field and in the post harvest conditions. In particular, maize is susceptible to infection by fungi belonging to *Fusarium* and *Aspergillus*. Several studies have assayed non-destructive, spectral methods to detect fungal contamination and toxins on cereals. The aim of this work is the early detection of toxigenic fungi on whole maize kernels, the discrimination between healthy and diseased kernels and the determination of damage degree by using a Spectral Imaging -based method. A desktop spectral scanner equipped with an imaging based spectrometer ImSpector-Specim V10, working in the visible-near infrared spectral range (400-1000 nm) was used in the experiments. Four pure toxigenic fungal strains were imaged, *A. parasiticus*, *A. flavus*, *A. niger* and *F. graminearum*. The results obtained indicate that spectral imaging can discriminate between different fungal strains, producing a specific spectral fingerprint for each one. These fungi have been also inoculated on maize kernels and were imaged from 24 h to 7 days of growth. Changes in reflectance of kernels were observed during fungal growth. Spectral imaging could be able to discriminate maize kernels infected with toxigenic fungi from uninfected controls; this technique could be used for an early detection of toxigenic fungi on cereals.

**PCR IDENTIFICATION OF *FUSARIUM* SPECIES ISOLATED FROM AGRICULTURAL SOILS IN ESKISEHIR PROVINCE.** R. Demirel<sup>1</sup>, N. Yılmaz Sarıözlü<sup>1</sup> and S. İlhan<sup>2</sup>. <sup>1</sup>Department of Biology, Faculty of Science, University of Anatolia, 26470, Eskisehir, Turkey. <sup>2</sup>Department of Biology, Faculty of Science and Art, University of Eskisehir Osmangazi, 26480 Eskisehir, Turkey. E-mail: rasime.demirel@gmail.com

*Fusarium* species are important plant pathogens around the world. Head blight and crown rot disease of cereals caused by these species are responsible for large economic losses due to reduction in seed quality and contamination of grain with their mycotoxins. This paper presents data on the PCR identification of *Fusarium* species isolated from agricultural soils from Eskisehir. Nine *Fusarium* species were isolated from 56 soil samples. DNA of these *Fusarium* species were purified by glass beads and vortexing extraction method and used for PCR amplification with universal fungal specific primers. The internal transcribed spacer (ITS) regions of ribosomal DNA were sequenced by CEQ 8000 Genetic Analysis System. The ITS-5.8S sequences obtained in this study were compared with those deposited in the GenBank Database.

**TOWARDS DEVELOPMENT OF DNA-BASED DIAGNOSTICS FOR PEA WILT PATHOGEN *FUSARIUM OXYSPORUM* f. sp. *PISI*.** K. Dev Sharma and A. Sharma. Advanced Centre of Hill Bioresources and Biotechnology, CSK Himachal Pradesh Agricultural University, Palampur, 176062 HP, India. E-mail: kml1967@rediffmail.com

*Fusarium oxysporum* f. sp. *pisi*, a vascular wilt pathogen of peas, is world wide in occurrence and causes large scale yield losses. The complexity associated with its correct identification by traditional methods necessitates the use of molecular techniques for its diagnosis. In an effort to develop DNA-based assays for its identification, we PCR amplified and sequenced 28S rDNA, intergenic spacer and internal-transcribed spacers regions of *F. oxysporum* f. sp. *pisi* and other fungi. Sequence analysis clustered four *formae speciales* (*pisi*, *vasinfectum*, *ciceris* and *udum*) of *F. oxysporum* into one group whereas *F. solani* isolates formed the second and *Phoma pinodella*, the third group. The analysis also revealed intra-*formae speciales* variability among *F. oxysporum* f. sp. *pisi* isolates. PCR-RFLP of the amplified products using 23 restriction enzymes was also carried out to identify *F. oxysporum* f. sp. *pisi* specific markers. PCR-RFLP of 28S rDNA region with restriction enzyme *MvaI* revealed a 370 bp band that could be the potential marker for diagnosis of *F. oxysporum* f. sp. *pisi* as it was absent in other root and stem invading pathogens of pea, three other *formae speciales* of *F. oxysporum* and non-pathogenic *F. solani*. The confirmation of the diagnostic ability of this marker by incorporating more isolates of the pathogen and other fungi is in progress.

**IDENTIFICATION OF A *FUSARIUM* sp. ASSOCIATED WITH CROWN ROT OF *XANTHORRHOEA* spp. IN TASMANIA.** M. Glen<sup>1</sup>, Z.Q. Yuan<sup>2</sup>, W. Dunstan<sup>3</sup>, T. Rudman<sup>4</sup> and C.L. Mohammed<sup>1,5</sup>. <sup>1</sup>CSIRO Forest Biosciences, Private Bag 12, Hobart, 7001 Tasmania, Australia. <sup>2</sup>Department Primary Industry and Water, 13 St John's Avenue, New Town 7008, Tasmania, Australia. <sup>3</sup>Murdoch University, South Street, Murdoch 6150, WA, Australia. <sup>4</sup>Department of Primary Industry and Water, GPO Box 44, Hobart 7001, Tasmania, Australia. <sup>5</sup>Tasmanian Institute of Agricultural Research, University of Tasmania, Private Bag 12, Hobart 7001, Tasmania, Australia. E-mail: Morag.Glen@csiro.au

In 2006, a crown rot and dieback of *Xanthorrhoea* spp. was ob-

served in coastal heath-land in NE Tasmania. Disease symptoms were observed at all levels in the landscape, from the verges of wetlands to the top of dry dunes and on northerly and southerly aspects. However, symptoms appear to be largely restricted to an area burnt in 2005. *Xanthorrhoea* spp. had also flowered prolifically in this area in 2006 while flowering was minimal outside the burnt area. Diseased and dead plants are dispersed widely through the *Xanthorrhoea* population, though small clusters occasionally occur. This pattern of disease differs markedly from the disease fronts and clustering of dead plants observed in *Phytophthora cinnamomi* infested *Xanthorrhoea* populations. A fungus isolated from the diseased plants was identified morphologically as belonging to the *Fusarium subglutinans* group, using Burgess's key. However subsequent production of chlamydospores contradicted this diagnosis. The fungus forms oval, elliptical or allantoid, or sometimes spindle-shaped microconidia in false-heads. Macroconidia are slender, falcate, 3-5-8 septate and thin-walled. It occasionally forms thick-walled, individual chlamydospores. This corresponds to the description of *F. babinda*, which was originally isolated from soil and has not been linked to plant disease. Sequences from the elongation factor and histone genes will be compared to sequences from *F. babinda* to confirm identification.

**SURVEY OF DISEASES CAUSED BY *FUSARIUM* spp. ON PALMS IN THE CANARY ISLANDS.** J. Hernández-Hernández<sup>1</sup>, A. Espino<sup>2</sup>, J.M. Rodríguez-Rodríguez<sup>3</sup>, A. Pérez-Sierra<sup>4</sup>, M. León<sup>4</sup>, P. Abad-Campos<sup>4</sup> and J. Armengol<sup>4</sup>. <sup>1</sup>ICIA, Departamento de Protección Vegetal, Carretera. El Boquerón, s/n, Apdo. 60, Valle Guerra, La Laguna 38270, Tenerife, Spain. <sup>2</sup>Laboratorio de Sanidad Vegetal, Consejería de Agricultura Ganadería y Pesca, Gobierno de Canarias, Carretera El Boquerón, s/n, Valle Guerra, La Laguna 38270, Tenerife, Spain. <sup>3</sup>Laboratorio de Fitopatología, Granja Agrícola, Cabildo de Gran Canaria, Ctra. Del Norte, Km. 7,5, 35400, Las Palmas de Gran Canaria, Gran Canaria, Spain. <sup>4</sup>Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: aperesi@eaf.upv.es

Between 2006 and 2007, palms showing symptoms of wilt and dieback were surveyed both in gardens and natural palm groves in the Canary Islands (Spain). Isolations from affected tissues of the crown, leaves and vascular fragments of symptomatic plants were performed on PDAS. After incubation, the *Fusarium* spp. colonies obtained were single spored and transferred to PDA and SNA for morphological identification. Additionally, the identification of *F. oxysporum* f. sp. *canariensis* was confirmed by PCR with specific primers HK66 and HK67 which amplify a fragment of 567 bp. *Fusarium* wilt caused by *F. oxysporum* f. sp. *canariensis* was found on 54 *Phoenix canariensis* and was widely distributed in four islands: Gran Canaria, Fuerteventura, La Palma and Tenerife. *F. proliferatum* was found on sixteen palms (11 *P. canariensis*, 2 *P. dactylifera*, 2 *Roystonea regia* and 1 *Vetchia joanis*) located in Gran Canaria and Tenerife. In all cases, both *Fusarium* species were found only on diseased palms from gardens, but not from natural palm groves. These results show the importance of *Fusarium* wilt of *P. canariensis* in the Canary Islands and report the presence of *F. proliferatum* affecting different palm species.

**EXPLORING THE DISTRIBUTION, PHYLOGENY AND POTENTIAL FOR EXPLOITATION IN DIAGNOSTICS OF THE *TRI5*, *TRI4* AND *TRI101* GENES IN *FUSARIUM* spp.** R. Kristensen, R.F. Fristad and A. Holst-Jensen. National Veterinary Institute, Section of Mycology, P.O. Box 8156 Dep., 0033 Oslo, Norway. E-mail: ralf.kristensen@vetinst.no

A central problem in design of molecular diagnostics is that of target coverage. Multiplexing is a challenge in itself, but in *Fusarium* the lack of concordance between biologically important features and species phylogeny complicates assay development further. Although desirable, it has therefore proven impossible to develop diagnostic probes or primers that single-handedly can identify species and simultaneously reveal their trichothecene mycotoxin synthetic abilities. We recently published two multiplex detection assays targeting a house-keeping gene by microarray and SNP analysis, respectively, illustrating this problem. Phylogenetic analyses of the trichothecene gene clusters have revealed discordance with species phylogeny. The B-trichothecene chemotype polymorphism seems to be transspecific and has been maintained through multiple speciation events. The non clustered *TRI101* gene has been found in non-trichothecene producing *Fusarium* species both as a functional and as a non-functional gene. So far there are no similar reports concerning genes from one of the two trichothecene gene clusters. In a quest to reveal some of the phylogenetic relationships of the *TRI5*, *TRI4* and *TRI101* genes, a range of both trichothecene (type A and type B) and putative non-trichothecene producing *Fusarium* species have been explored. The results of this study will be presented and the possibilities for exploitation of these genes in diagnostics will be discussed.

**ETIOLOGY OF MANGO MALFORMATION AND PCR DETECTION OF ITS CAUSAL AGENTS IN BRAZIL.** C.S. Lima<sup>1</sup>, S.S. Costa<sup>1</sup>, M.A. Campos<sup>2</sup>, J.F. Leslie<sup>3</sup> and L.H. Pfenning<sup>1</sup>. <sup>1</sup>Departamento de Fitopatologia, Universidade Federal de Lavras, 37200000 Lavras, MG, Brazil. <sup>2</sup>Departamento de Biologia, Universidade Federal de Lavras, 37200000 Lavras, MG, Brazil. <sup>3</sup>Department of Plant Pathology, Kansas State University, 66506-5502 Manhattan, KS, USA. E-mail: ludwig@ufla.br

This study aimed at characterizing the *Fusarium* population associated with mango malformation disease in Brazil through AFLP analysis, sequencing of portions of the *tef1* and *tub2* genes, crossing of strains on carrot agar, and pathogenicity tests. Additionally, sequences of *tef1* were used to design species-specific primers for PCR detection of the causal agents in plant tissue. Three clusters of isolates were observed in the AFLP analysis. The majority of the isolates fell in the first cluster, while the second contained isolates of *F. sterilibyphosum* from Brazil and South Africa. The third cluster grouped isolates of *F. mangiferae* from Egypt, India, South Africa, and USA. A unique Brazilian clade in the *Gibberella fujikuroi* species complex was observed in the phylogenetic analysis. Its isolates were also cross-fertile, producing a typical *Gibberella* teleomorph. All the crosses between isolates of the Brazilian clade and isolates of *F. sterilibyphosum* and *F. mangiferae* were not fertile. Isolates of the Brazilian clade and *F. sterilibyphosum* were pathogenic to inoculated mango plants. A primer set that amplifies a 380 bp fragment from isolates of the Brazilian clade and *F. sterilibyphosum* was selected. Another fragment of 217 bp in size was obtained with a specific primer set to *F. mangiferae*. Specificity and sensitivity of primer sets were evaluated. The first primer set detected the presence of the causal agents of malformation in infected trees and in greenhouse plants artificially inoculated.

Work supported by Fundação de Apoio à Pesquisa do Estado de Minas Gerais FAPEMIG (CAG 1096/03).

**MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM* F. SP. *MELONIS* RACE 2.** L. Luongo<sup>1</sup>, S. Vitale<sup>1</sup>, A.

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*Fusarium oxysporum* f. sp. *melonis* (Fom) causes Fusarium wilt of melon which is an economically important disease worldwide. Four races of the pathogen have been designated 0, 1, 2, and 1,2 based on the interaction between two resistance genes and variants of the pathogen. Two dominant resistant genes *Fom-1* and *Fom-2* provide resistance to races 0 and 2, and races 0 and 1, respectively. Race 1,2 overcomes these resistance genes. A collection of 48 isolates of Fom representative of all four races was analyzed by several molecular techniques in attempt to characterize the *forma specialis* and the race. Genetic variation among races was evidenced by random amplified polymorphic DNA (RAPD) and translation elongation factor 1-alpha partial sequence (TEF). Among the 89 RAPD primers tested the polymorphic profiles of 23 primers originated a dendrogram in which race 2 clearly differentiated from all other races. Highly polymorphic bands of 3 primers were chosen. They were cloned and sequenced. The corresponding sequences will be used for specific primers design and further information on Fom race 2 differentiation, which is inferred to have a different origin from races 1 and 1,2. RAPD markers proved to be a valid tool for the characterization of races within Fom, as already reported for other *formae speciales* of *F. oxysporum*, namely *ff.spp. pisi*, *vasinfec-tum* and *dianthi*.

**POPULATION DYNAMICS OF FUSARIUM HEAD BLIGHT ISOLATES IN SOUTH AFRICA. A. Minnaar-Ontong<sup>1</sup>, L. Herselman<sup>1</sup>, W.M. Kriel<sup>1</sup> and J.F. Leslie<sup>2</sup>.** <sup>1</sup>Department of Plant Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, South Africa. <sup>2</sup>Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas 66506-5502, USA. E-mail: MinnaarA.sci@ufs.ac.za

The genus *Fusarium* includes more than fifty species and the number is still increasing. *Fusarium* spp. all over the world includes toxin producers and plant pathogens, causing diseases on a wide range of plant species. One of the most important diseases is Fusarium head blight (FHB) of wheat, an economically devastating disease that reduces grain quality and yield. Mycotoxins produced by FHB-causing species, can be harmful to both humans and livestock. Species implicated in the disease include *F. graminearum*, *F. culmorum*, *F. cerealis* and *F. avenaceum*. Effective disease control depends on an understanding of the etiology of the disease. The aim of this study was to combine different methods (morphological, molecular and chemotoxicity) to give insight into hidden population dynamics of FHB-causing species in South Africa. Samples were collected from FHB infected wheat heads from the principle irrigation areas of South Africa, plated out and morphologically identified based on single-spore cultures. Chemotypes were characterised using DON and NIV specific PCR primers. Molecular characterisation of *Fusarium* isolates was done using amplified fragment length polymorphism (AFLP) analysis, due to its highly discriminatory power. AFLP analysis was done using three different primer pair combinations on 860 strains of morphologically identified *Fusarium* isolates. Analyses detected genetic variation among and within *Fusarium* spp. in South Africa. This indicated that AFLP analysis is a successful tool in the molecular identification of *Fusarium* isolates and gave useful insights into the population dynamics of FHB isolates in South Africa.

**MOLECULAR DETECTION OF *FUSARIUM VERTICILLIOIDES*, *F. PROLIFERATUM* AND *F. SUBGLUTINANS* USING POLYMERASE CHAIN REACTION. M. Moretti, G. Venturini, G. Assante and A. Vercesi.** Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: maddalena.moretti@unimi.it

*Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans*, the aetiological agents of corn ear rots, are morphologically very similar, but differ for their toxigenic capabilities. In fact, only *F. verticillioides* and *F. proliferatum* are strong fumonisin producers. Therefore a reliable and rapid method is required to detect and distinguish these three species in maize kernels. Four different primer pairs, namely VER1/2 and VERTF1/2 species-specific for *F. verticillioides*, PRO1/2 for *F. proliferatum* and SUB1/2 for *F. subglutinans*, were tested on several strains belonging to 16 well known *Fusarium* species commonly associated with corn. VER1/2 and VERTF1/2 detected not only *F. verticillioides* but also *F. nygamai* and *F. napiforme*. Moreover, VER1/2 amplified the target DNA of *F. equiseti*. PRO1/2 was strictly species-specific for *F. proliferatum*. SUB1/2 detected both *F. subglutinans* and *F. nygamai*. Molecular analysis carried out on corn samples naturally contaminated with *F. verticillioides* and/or *F. proliferatum* gave inconsistent results. Therefore, the proposed primer pairs were able to detect *F. verticillioides*, *F. proliferatum* and *F. subglutinans* but should be further optimized to be used as species-specific diagnostic tools especially where *F. nygamai*, *F. napiforme* and *F. equiseti* are likely to be found as corn contaminants.

**A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE SPECIFIC DETECTION OF *FUSARIUM GRAMINEARUM*. L. Niessen and R.F. Vogel.** Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Weibensteinstrasse 16, 85350 Freising, Germany. E-mail: niessen@wzw.tum.de

Loop-mediated isothermal amplification (LAMP) of DNA is a simple, cost effective, and rapid novel method for the specific detection of genomic DNA using a set of 8 primers binding specifically to different regions of a target gene, and a thermophilic DNA polymerase from *Bacillus stearothermophilus*. The method has been applied, since 2000, in various assays for the diagnosis of bacterial and viral infections of humans and animals, sexing of bovine and swine embryos, and in the detection of bacteria from environmental samples. Only recently the first applications for fungal organisms (*Phytophthora ramorum*, *Brettanomyces* spp.) were published. We have developed a LAMP assay for the specific detection of *Fusarium graminearum* based on the *gaoA* gene. The assay was optimized for rapidness, specificity, and sensitivity. Analysis was completed within 20 minutes, including detection. Amplified product was detected in a simple fluorometric and colorimetric test with no agarose gel electrophoresis necessary for product visualization. The limit of detection was found to be less than 100 fg of target DNA per reaction. The assay will be applied to the simple and rapid detection of *F. graminearum* in naturally and artificially infected wheat, barley and brewing malt.

**DIFFERENTIATION OF 12 *FUSARIUM* SPECIES ISOLATED FROM SUGAR BEET APPLYING RESTRICTION FRAGMENT ANALYSIS OF PCR AMPLIFIED TRANSLATION ELONGATION FACTOR 1 $\alpha$  GENE FRAGMENT. E. Nitschke and M. Varrelmann.** Institute of Sugar Beet Research, Holtenser Landstrasse 77, 37079 Göttingen, Germany. E-mail: varrelmann@ifz-goettingen.de

Sugar beet in Europe, grown in wheat and maize crop rotations and subsequently pile-stored for several weeks possesses attributes to be attacked by saprophytic as well as pathogenic *Fusarium* spp. during growth and storage. As *Fusarium* species display variable toxigenic potential, accurate identification is necessary to estimate the impact of different species on mycotoxin accumulation in sugar beets, especially regarding their processing to beet fibers as forage. For the numerous species being isolated from sugar beets a tool for reliable identification based on sequence information of the translation elongation factor 1 $\alpha$  (TEF-1 $\alpha$ ) gene was developed. One-hundred isolates from different species (*F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. redolens*, *F. sambucinum*, *F. solani*, *F. tricinctum* and *F. venenatum*) were obtained from sugar beet at different developmental and storage stages from locations worldwide. Database sequences (*Fusarium*-ID) from additional species (*F. sporotrichioides*, *F. poae*, *F. torulosum*, *F. hostae*, *F. subglutinans*, *F. verticillioides*) were included in the analyses. Molecular sequence analysis of the partial TEF-1 $\alpha$  gene fragment revealed sufficient variability to differentiate between the *Fusarium* species resulting in species dependent separation of the analysed isolates. This interspecific divergence could be translated into a PCR-RFLP assay using only two independent restriction digests for the differentiation of 17 out of 18 species. The developed TEF-1 $\alpha$  based PCR-RFLP assay was able to identify and differentiate a sugar beet derived broad spectrum of *Fusarium* species including pathogenic species with relevance for cereals in crop rotations as well as saprophytic living species.

**REAL-TIME PCR ASSAYS FOR *FUSARIUM VERTICILLIOIDES* AND *F. PROLIFERATUM*.** S. Nutz, K. Döll and P. Karlovsky. Georg-August-University Goettingen, Molecular Phytopathology and Mycotoxin Research Unit, Grisebachstrasse 6, 37077 Göttingen, Germany. E-mail: pkarlov@gwdg.de

*Fusarium verticillioides* and *F. proliferatum* are important pathogens of maize world-wide. Maize disease caused by *Fusarium* spp. leads to severe losses in yield and to the accumulation of mycotoxins. *F. verticillioides* and *F. proliferatum*, belonging to *Gibberella fujikuroi* species complex, are the most prolific producers of fumonisins. These mycotoxins cause severe animal diseases such as equine leucoencephalomalacia in horses and pulmonary oedema in pigs. Fumonisin B1 is a proven carcinogen in rodents and a suspected carcinogen in humans. Apart from diseases known as *Fusarium* ear rot and ear mold, symptomless infections by *Fusarium* species of the *Liseola* section were reported in maize. Therefore, visually rated symptoms are not a reliable predictor of mycotoxin contamination. Real-time PCR assays allows species-specific quantification of *Fusarium* biomass in the plant tissue, which is a crucial requirement for the assessment of fungal virulence. Furthermore, quantitative characterization of plant colonization is important in epidemiological studies. Real-time PCR (qPCR) offers a precise, fast and relatively inexpensive method for this purpose. The specificity of the assays developed in this work was tested with a wide range of fungi species, including all relevant *Fusarium* spp. The assay was validated with artificially contaminated maize samples.

**REAL-TIME PCR PROTOCOL FOR *FUSARIUM POAE* DETECTION IN WHEAT.** A. Prodi<sup>1</sup> and P. Karlovsky<sup>2</sup>. <sup>1</sup>Department of Agroenvironmental Science and Technology, Area of Plant Pathology, Alma Mater Studiorum University of Bologna, Viale

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Durum wheat (*Triticum durum* Desf.) is one of the most widely grown crops in Italy. Apart from supplying the food chain with an essential raw material, growing durum wheat provides a vital source of income for Italian farmers. One of the major challenges in wheat production is a fungal disease called *Fusarium* head blight (FHB). Several species of *Fusarium* are involved in FHB, most prevalent in Italy being *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. The frequency of occurrence of *F. poae* in durum wheat samples collected from Emilia-Romagna fields (Northern Italy) during the last ten years grew from 9% in 1998 to 23% in 2007. Similar development has been reported from other areas of Italy. Although the pathogenicity of *F. poae* and its role in the etiology of FHB has not been conclusively determined, the increasing incidence of the fungus in durum wheat is alarming because of its ability to produce mycotoxins of the trichothecene group. The aim of the present study was to establish a method for the quantification of the biomass of *F. poae* suitable for epidemiological studies which involve a large number of wheat samples. A fast and cost-effective real-time PCR protocol based on the fluorescent dye SYBR Green was developed and validated.

**TESTING BARCODE MARKERS IN ASCOMYCOTA: INTRON DISTRIBUTION ALONG MITOCHONDRIAL GENES.** M. Santamaria<sup>1</sup>, S. Vicario<sup>1</sup>, C. Scazzocchio<sup>3</sup>, S. Somma<sup>4</sup>, A. Moretti<sup>4</sup> and C. Saccone<sup>1,2</sup>. <sup>1</sup>Istituto di Tecnologie Biomediche del CNR, Sede di Bari, 70126 Bari, Italy. <sup>2</sup>Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi, 70126 Bari, Italy. <sup>3</sup>Institut de Génétique et Microbiologie, UMR 8621 CNRS, Université Paris-Sud (XI) and Institut Universitaire de France, Bâtiment 409, Centre d'Orsay, 91405, Orsay Cedex, France. <sup>4</sup>Institute of Sciences of Food Production, CNR, 70126 Bari, Italy. E-mail: cecilia.saccone@itb.cnr.it

Fungi require a rapid, standardized, and cost-effective identification system, owing to the frequent lack of distinctive morphological characters and the preponderance of microscopic species. The present work would contribute to the development of effective species molecular markers in Ascomycota, a phylum with a large economic impact both as pathogens and as beneficial organisms involved in the quality and safety of agri-food products. Currently, the molecular identification of species in fungi is based primarily on nuclear DNA, but the potential use of mitochondrial markers has also been considered, notwithstanding possible complications in the PCR-based genetic divergence surveys due to the presence of mobile introns in almost all mitochondrial genes of these organisms. The aim of this work is to find one or more mitochondrial gene regions free from introns and large enough to be proposed as species molecular marker (barcode) candidates. Therefore, the distribution and size of introns in 11 protein coding genes and 2 rRNA coding genes, belonging to Ascomycota mitochondrial DNA and available in public database, have been investigated using two bioinformatic methodologies: a Blast-based approach and a Query-based approach. Our results confirm the trend in Ascomycota: only few sequences seem to be free from introns and, among them, ND6, ND2 and ND4 genes could really be the best barcode candidates. A procedure of amplification, sequencing and genetic divergence analysis of these genes is being carried out in about 50 populations of *Fusarium* in order to investigate their adequacy to be good species markers.

**PATHOGENIC AND MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM* f. sp. *ALBEDINIS* STRAINS, CAUSAL AGENT OF THE BAYOUD DISEASE OF DATE PALM.** My. H. Sedra. 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. E-mail: sedramb@hotmail.com and mhsedra@yahoo.fr

Bayoud caused by *Fusarium oxysporum* f. sp. *albedinis* is uncontestedly the most serious and devastating fungal disease of the date palm (*Phoenix dactylifera* L.) in the world, with which it is difficult to cope. It is distributed in date-growing areas of North Africa, particularly in Morocco and Algeria where it has caused big losses. Since some last years, it was discovered in Mauritania and constitutes a veritable threat to neighboring countries and to date producer countries in the world. The diagnosis of the disease showed that it presents typical and atypical symptoms. Sometimes, the disease is expressed in association with other diseases on the same date palm. Some pathogenic strains, although having different origin of isolation and showing different morphological, cultural aspects and molecular profiles, showed both pathogenic activity on date palm but with different levels of aggressiveness. The characterization of different strains isolated from soil, of date palm, of the Island Canary palm and plants so-called healthy carriers, permitted to put in evidence this diversity. This allows asking some questions on the origin of the disease and to open perspectives of applicable research.

**USE OF GENETIC FINGERPRINTING TO STUDY THE DIVERSITY IN *FUSARIUM OXYSPORUM* f. sp. *CICERIS*, CHICKPEA WILT PATHOGEN.** M. Sharma, S. Pande, R. Varshney, S. Kannan and D. Hoisington. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502 324, Andhra Pradesh, India. E-mail: mamta.sharma@cgiar.org

Fusarium wilt caused by *Fusarium oxysporum* Schl. Fr. f. sp. *ciceris* (Padwick) [FOC] is a major disease of chickpea (*Cicer arietinum* L.) and causes up to 100% losses under favourable environmental conditions. FOC have extreme phenotypic and genotypic variability and frequent reoccurrence of its virulent forms have affected chickpea production worldwide and exhausted valuable genetic resources. Forty-eight isolates of FOC collected from different chickpea growing regions in India were analysed for virulence and genetic variations. Characterization of virulence and genetic variation was based on their pathogenicity and Amplified Fragment Length Polymorphism (AFLP), respectively. Out of 48 isolates 41 were found virulent and seven avirulent. Disease development indicated that the virulent isolates differ in their aggressiveness, while there was no apparent correlation between geographical origin and aggressiveness of the isolates. A total 339 bands were scored following selective amplification with five *Eco*RI and *Mse*I primer combinations E-TC/M-CAT, E-TC/M-CAC, E-AC/M-CAG, E-TA/MCAG, E-TA/M-CAG, out of which 331 bands were polymorphic. UPGMA cluster analysis and principle coordinate analysis separated the 48 isolates distinctly into two major groups; virulent and avirulent. Among the virulent isolates, a total 258 bands were scored out of which 202 were polymorphic and the isolates could be clustered into six major groups at 0.77 genetic similarities. Region specific grouping was observed with in few isolates. The results of the present study provide evidence of the high discriminatory power of AFLP analysis, suggesting the applicability of this method to the molecular characterization of *F. oxysporum* f. sp. *ciceris*.

**THE OCCURENCE OF NIVALENOL-PRODUCING *FUSARIUM* ISOLATES ON WHEAT IN THE CZECH REPUBLIC.** T. Sumíková<sup>1,2</sup>, J. Chrpová<sup>1</sup>, S. Sykorová<sup>1</sup> and V. Šíp<sup>1</sup>. <sup>1</sup>Crop Research Institute, Drnovská 507, CZ-161 06, Prague 6, Czech Republic. <sup>2</sup>Czech University of Life Sciences, Kamycká 129, CZ-165 21, Prague 6, Czech Republic. E-mail: sumikova@vurv.cz

Fusarium head blight is one of the most important diseases of wheat in the Czech Republic. The infections occur every year and can cause yield reduction and mycotoxin contamination of grain. The most important mycotoxins are trichothecenes such as deoxynivalenol (DON) and nivalenol (NIV). Recently PCR assays to trichothecene biosynthetic genes (*Tri* genes) were developed to characterize the mycotoxin chemotypes of *Fusarium* species causing head blight. The aim of this study was to detect the chemotypes of 119 *Fusarium graminearum* and *F. culmorum* isolates from infected wheat ears taken in years 2003-2005 from different areas of the Czech Republic. Molecular markers for detection *Tri7* and *Tri13* genes were used. Only three producers of nivalenol were detected. Eighteen isolates including these nivalenol producers were used for infection field tests to score their aggressiveness and the content of DON by ELISA method. Low content of DON and high aggressiveness was found out in all samples infected by nivalenol producers.

Work supported by project No. QG50076 of the Ministry of Agriculture of the Czech Republic.

**REAL-TIME PCR QUANTIFICATION OF *FUSARIUM* DNA AND CORRELATION TO MYCOTOXIN LEVELS IN FINNISH CEREALS.** T. Yli-Mattila<sup>1</sup>, T. Lahtinen<sup>1</sup>, S. Rämö<sup>2</sup>, M. Kokkonen<sup>3</sup>, A. Rizzo<sup>3</sup>, M. Jestoi<sup>3</sup> and V. Hietaniemi<sup>2</sup>. <sup>1</sup>Laboratory of Plant Physiology and Molecular Biology, University of Turku, Turku, Finland. <sup>2</sup>MTT Agrifood Research Finland, Laboratories, Finland, Jokioinen, Finland. <sup>3</sup>Finnish Food Safety Authority (EVI-RA), Department of Animal Diseases and Food Research, Unit of Chemistry and Toxicology, Helsinki, Finland. E-mail: tymat@utu.fi

Our work is part of a project aiming at monitoring mycotoxin and *Fusarium* levels in Finland during 2005-2006 (Finmyco). A highly significant correlation was found between *Fusarium graminearum* DNA and deoxynivalenol (DON) levels in Finnish oats, barley and spring wheat. In barley *F. culmorum* plays a more important role in DON production than in spring wheat or oats, because the correlation was higher in barley with the combined *F. graminearum*/*F. culmorum* DNA. A significant correlation was also found between *F. avenaceum* DNA and enniatins in spring wheat, barley and oats and between *F. avenaceum* DNA and moniliformin (MON) in barley and spring wheat. The correlation between *F. langsethiae*/*F. sporotrichioides* DNA and HT-2+T-2 levels was highly significant in oats, in which the highest HT-2+T-2 levels were found. The correlation between *F. poae* DNA and NIV was significant in both barley and oats. The possibility to use and further develop these qPCR methods for screening grain samples in order to reliably distinguish grain samples with suspected high mycotoxin levels in different cereals will be discussed. The coefficient of determination ( $R^2$ ) between *F. graminearum* DNA and DON was about 0.50 (0.49-0.61), when DNA was extracted from the grain surfaces. Higher  $R^2$  values (0.54-0.88) between *F. graminearum* DNA and DON in oats, barley and spring wheat were obtained when DNA was extracted from ground grains. The  $R^2$  values between *F. langsethiae*/*F. sporotrichioides* DNA and HT-2+T-2 levels and between *F. avenaceum* DNA and enniatins were also clearly higher when DNA was extracted from ground grains.