



GENETIC AND PHYSIOLOGICAL DIVERSITY OF *FUSARIUM OXYSPORUM* ISOLATES FROM TOBACCO FIELDS IN SPAIN. F.M. Alves-Santos¹, D. Martínez-Bermejo¹, M.C. Rodríguez-Molina² and J.J. Diez¹. ¹Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Escuela Técnica Superior de Ingenierías Agrarias de Palencia, Avenida de Madrid 57, 34071 Palencia, Spain. ²Centro de Investigación 'La Orden-Valdesequera', Consejería de Infraestructuras y Desarrollo Tecnológico, 06187 Guadajira, Badajoz, Spain. E-mail: fmalvess@pvs.uva.es

Several *formae speciales* of *Fusarium oxysporum* are pathogenic to tobacco plants. Different authors have classified those isolates as a *forma specialis* or a race within on the basis of the severity of disease and host specificity. *Fusarium* wilt of tobacco has been recorded in the last years in Extremadura (Central Spain). *F. oxysporum* was isolated from symptomatic plants and characterized as *forma specialis batata*. The aim of our study was to characterize these *F. oxysporum* populations. For this purpose, the *in vitro* spore production and growth and the virulence (severity of disease) have been tested. Although all isolates behaved as pathogen, the virulence of isolates was different. The differences in growth could not be correlated with other characteristics but the two isolates with scarce spore production have also behaved as the weakest pathogen. We have analyzed intergenic spacer (IGS) region polymorphism of ribosomal DNA and random amplified polymorphic DNA (RAPD) markers to assess the genetic diversity within *F. oxysporum* isolates. These molecular analyses showed two major groups with different physiological capabilities that could reflect two different lineages. One group was characterized by medium-high sporulation, high virulence and the same IGS-RFLP pattern. The other group was more heterogeneous featuring low-medium sporulation and variable virulence and growth. This experimental approach to pathogen population could be a good starting point for further studies including non-pathogenic isolates and a larger number of pathogen that could clarify if there are two or more genetic lineages.

PHENOTYPIC AND GENOTYPIC DIVERSITY IN *FUSARIUM CIRCINATUM*. M. Berbegal¹, A. Pérez-Sierra¹, J. García-Jiménez¹, J. Armengol¹ and M.M. Jiménez-Gasco². ¹Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. ²Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA. E-mail: mobermar@etsia.upv.es

A severe outbreak of pitch canker caused by *Fusarium circinatum* was first reported in 2005 on *Pinus* spp. in northern Spain. Since then, new foci of the disease have been detected both in nurseries and forest plantations. *MAT-1* and *MAT-2* isolates were found in Spain and morphological differences were linked to them. Isolates of *F. circinatum* from the International Collection of the *Fusarium* Research Centre (The Pennsylvania State University), including reference testers obtained from the CBS, were studied to confirm the association between morphological characters and mating type. In general, *MAT-1* isolates showed the typical sterile coiled hyphae, but sterile hyphae from *MAT-2* isolates were not coiled. Furthermore, the genotypic diversity of a representative set of 25 isolates from Spain, USA and South Africa, selected based on mating type, different geographical locations and hosts, was analyzed. Molecular characterization was performed using several different markers: the translation elongation factor 1-alpha gene (TEF), the entire intergenic region of the rRNA gene cluster (IGS) and nine sequence-characterized amplified polymorphic markers. The TEF gene genealogy grouped all the isolates together but three from USA, and three alleles were observed among the 25 iso-

lates studied using the nine polymorphic markers. All groups observed included isolates of both mating types. Results revealed a low genetic diversity and also suggest that sexual reproduction might be occurring in *F. circinatum* in Spain.

CHARACTERIZATION OF *FUSARIUM* SPECIES ON *SPARTINA ALTERNIFLORA* BY MORPHOLOGY, PATHOGENICITY, AND PHYLOGENY. W.H. Elmer and R.E. Marra. Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA. E-mail: Wade.Elmer@po.state.ct.us

Sudden vegetation dieback (SVD) was first noticed in 1999 in salt marshes along the Gulf and Atlantic coasts. It is characterized as a rapid loss of *Spartina alterniflora*, the major wetland grass. Affected sites become barren mudflats interspersed with remnant peat that shows no regrowth after several years. We investigated whether or not *Fusarium* pathogens were associated with declining plants at SVD sites from Maine to Georgia. A collection of over 100 isolates, cultured from *S. alterniflora*, fell morphologically into two major groups that could not be readily identified. Inoculation into stems of healthy *S. alterniflora* plants showed that one of two groups could be classified as mildly virulent and the other group was avirulent. DNA sequence data from 20 virulent isolates and 10 avirulent ones were obtained from partial DNA sequences of three genes: translation elongation factor 1-alpha (730 bp), calmodulin (700 bp), and β -tubulin (1319 bp). Published sequences from related species were included for purposes of rooting the resulting phylogenetic trees. Both Neighbor-Joining and Maximum Parsimony phylogenetic analyses of sequences, from all three genes, with bootstrap support for trees, demonstrated that the *Spartina* pathogens cluster as a clade distinct from any currently described species of *Fusarium*. We hypothesize that drought and rising sea levels have impacted these marshes and predisposed *S. alterniflora* to be more susceptible to this undescribed *Fusarium* epiphyte.

MULTI-GENE SEQUENCING AND MICROSATELLITE MARKERS REVEAL GENETIC DIVERSITY AMONG VEGETATIVE COMPATIBILITY GROUPS OF *FUSARIUM OXYSPORUM* f. sp. *CUBENSE*. G. Fourie¹, E.T. Steenkamp¹, T.R. Gordon² and A. Viljoen^{1,3}. ¹Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. ²Department of Plant Pathology, University of California, Davis, California, 95616, USA. ³Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. E-mail: gerda.fourie@fabi.up.ac.za

Fusarium oxysporum f. sp. *cubense* (*Foc*) is the causal agent of *Fusarium* wilt of banana. Various phenotypic and genotypic markers have divided *Foc* into two distinct clades, which suggest a polyphyletic origin. The objective of this investigation was to determine the relatedness among vegetative compatibility groups (VCGs) of *Foc* from these two clades. A total of 239 isolates were included that represents different VCGs, other *formae speciales* and non-pathogenic *F. oxysporum* isolates. Phylogenetic analysis using multi-gene sequences of two nuclear and two mitochondrial regions separated the isolates into the two main clades that both included *formae speciales* of *F. oxysporum* other than *Foc*. Measures of gene and genotypic diversity and overall genetic structure as assessed with microsatellite markers supported the idea that *Foc* comprise of clonal lineages. The results also demon-



strated that VCGs, in combination with their phylogenetic association, remains a powerful tool for characterizing *Foc* isolates. The mating type of all isolates was determined and isolates harbouring *MAT-1* and *MAT-2* genes were crossed, without success. However, the opposite mating types found in the respective lineages indicated a sexual origin for the *Fusarium* wilt fungus that could account for its polyphyletic nature. Furthermore, the hypothesis of recombination, tested with the index of association using microsatellite data, could not be rejected within some *Foc* lineages. Cultural and morphological variation of *Foc* was insufficient to suggest multiple species. However, the significance of the multiple lineages as well as genetic isolation of some of the lineages suggests that they most probably harbour phylogenetic species.

MOLECULAR GENETIC DIVERSITY AND VARIABILITY FOR AGGRESSIVENESS IN POPULATIONS OF THE WHEAT CROWN ROT FUNGUS *FUSARIUM PSEUDOGRAMINEARUM*. S.K. Gargouri, I. M'tat, L.G. Kammoun and M.R. Hajlaoui. *Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, Rue Hédi Karray, 2049 Tunis, Tunisia. E-mail: kammoun.samira@iresa.agrinet.tn*

Fusarium pseudograminearum (teleomorph: *Gibberella coronicola*), is one of the major pathogen causing wheat crown rot in the semi-arid area in Tunisia. In this study, 75 strains *F. pseudograminearum* representing five populations from Tunisia and one from a world collection were investigated for their molecular diversity. Thirty-four strains were further characterized for aggressiveness in a seedling assay. Mycotoxin-producing ability was tested by PCR using primer-pairs specific for the *Tri7* and *Tri13* genes. All the strains belonged to the DON chemotype. These strains were further characterized for their acetylated derivatives, using primer pair specific for the *Tri3* gene. All strains were classified as 3-AcDON. This information is of a particular concern since *F. pseudograminearum* can be also responsible of *Fusarium* head blight and the mycotoxin accumulation in the grains. The identification of mating type idiomorph was determined using diagnostic PCR primer for *MAT1-1* and *MAT1-2*. Both mating types were recovered from the same region and even from the same field, but 70% of the strains carried the *MAT1-2* gene. Restriction analysis of the nuclear ribosomal DNA (nrDNA) intergenic spacer region (IGS) revealed 11 haplotypes among the 75 strains. The analysis of population structure using the combined IGS and MAT data revealed that the total gene diversity ($H_t=0.108$) was mostly attributable to diversity within populations ($H_s=0.102$) and that the genetic differentiation among populations was low ($G_{st}=0.09$). Aggressiveness differed significantly within different isolates, and more than 80% of the Tunisian strains were highly aggressive, whereas more than 80% of the foreign isolates were poorly aggressive.

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THE HIDDEN DIVERSITY OF *COSMOSPORA* AND ITS *FUSARIUM* ANAMORPHS. T. Gräfenhan¹, H.I. Nirenberg² and K.A. Seifert¹. ¹*Biodiversity (Mycology & Botany), Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada.* ²*Julius-Kühn-Institute, Institute of Epidemiology and Pathogen Diagnostics, Königin-Luise-Strasse 19, 14195 Berlin, Germany. E-mail: graefenhant@agr.gc.ca*

The hypocrealean genus *Cosmospora* was resurrected by Rossman *et al.* (1999), mainly comprising members of the former *Nectria episphaeria* group. For almost a century, anamorphs of various hyphomycete genera were linked to different species of *Cosmospora*. These anamorphs include members of *Acremonium*, *Chaetopsina*, *Cylindrocladiella*, *Fusarium*, *Stilbella*, *Verticillium*, and *Volutella*. Thus, *Cosmospora* has long been assumed to be polyphyletic, but no formal taxonomic conclusions have been drawn. We analyzed *rpb2* and *acl1* gene sequences of more than 230 anamorph and teleomorph isolates from different geographic origin and substrates. The inferred phylograms reveal several polyphyletic groups. For example, *Cosmospora consors*, *C. chaetopsinae-penicillatae*, *C. desmazierii*, and *C. zealandica* are not closely related to the type species *C. coccinea*. The latter forms a distinct clade with greenish anamorph taxa and the *C. episphaeria/Fusarium aquaeductuum* var. *medium* complex. Other *Fusarium* anamorphs of *Cosmospora* spp., such as *F. ciliatum*, *F. epistromum*, *F. gigas*, *F. matuoi*, *F. sphaeriae*, several *F. merismoides* varieties, and entomogenous fusaria are phylogenetically distinct from the clade of the type species. We will discuss whether other generic names should be designated for these teleomorphs. Also, the known and accepted *Cosmospora-Fusarium* connections need to be reviewed critically. Interestingly, several *Cosmospora* species are characterized by obvious host specificity. For example, some *Cosmospora* spp. linked to members of *Fusarium* sect. *Macroconia* occur only on specific hosts such as *Buxus* or *Urtica*. A group of so far unidentified *Cosmospora* specimens seems to be host specific as well and may be closely related to *C. purtonii*.

TWO NEW *FUSARIUM* SPECIES AND A NEWLY RECOGNIZED PHYLOGENETIC LINEAGE. T. Gräfenhan¹, H.I. Nirenberg² and K.A. Seifert¹. ¹*Biodiversity (Mycology & Botany), Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada.* ²*Julius-Kühn-Institute, Institute of Epidemiology and Pathogen Diagnostics, Königin-Luise-Strasse 19, 14195 Berlin, Germany. E-mail: graefenhant@agr.gc.ca*

Two infrequently reported members of *Fusarium* sect. *Discolor* are only known from subtropical and tropical regions, *viz.* *F. bubaricum* and *F. sublunatum*. Along with the two new tropical species reported here, they form a basal lineage in the *Gibberella* clade. *Fusarium coeruleum* and the *F. stilboides* / *F. lateritium* var. *longum* complex are sister lineages of the new lineage. Morphologically, the new species are characterized by similar sporodochial conidia to those seen in *F. bubaricum* and *F. sublunatum*. The predominantly 5-septate macroconidia have a hooked apical cell and a distinctly pedicellate foot cell. Usually chlamydospores are formed early and are occasionally abundant in the aerial mycelium. *Fusarium* sp. 1, which often produces blue sporodochia, was isolated from *Abutilon theophrasti* (*Malvaceae*) in Canada (Ontario), USA (Wisconsin), and from soil from New Caledonia. This species is shown to be pathogenic to *A. theophrasti*. *Fusarium* sp. 2 was isolated only from soil from Guadeloupe.

PHYLOGENETIC ANALYSIS OF *FUSARIUM OXYSPORUM* ISOLATED FROM THE TISSUES AND RHIZOSPHERE OF *LYCOPERSICON* spp. K. Inami¹, M. Kawabe², A. Okabe¹, N. Ishikawa¹, T.L. Peever³, M. Kodama⁴, T. Teraoka⁵ and T. Arie⁵. ¹*Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TU-AT), Tokyo, Japan.* ²*The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama, Japan.* ³*Department of*

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Fusarium oxysporum f. sp. *lycopersici* (FOL) is a soilborne pathogen causing vascular wilt disease of tomato. *F. oxysporum* is also a ubiquitous soil resident and non-pathogenic strains (NPF) are frequently isolated from tomato tissues and rhizosphere. Our working hypothesis is that NPF has acquired pathogenicity factors during their non-pathogenic association with tomato allowing FOL strains to emerge. The origin of edible tomato (*Lycopersicon esculentum*) is considered to be in the Andean Plateau and several wild *Lycopersicon* spp. can be found there. Several *Lycopersicon* spp. were transported to Mexico where cultivated *L. esculentum* is thought to have been selected. After the 16th century, *L. esculentum* was spread worldwide. To address our hypothesis, we sampled fungi from wild, symptomless *Lycopersicon* spp. and the soil in which these plants were grown in Chile, Ecuador, and Mexico. From tissues and rhizosphere soil we obtained approximately 200 *F. oxysporum* isolates. Pathogenicity of these isolates was tested and all did not produce wilt symptoms on tomato. Approximately 600 base pairs of the nuclear ribosomal intergenic spacer were sequenced from each isolate and used to estimate a phylogeny which included FOL and other *formae speciales* of *F. oxysporum*. Isolates sampled from *Lycopersicon* spp. and rhizosphere soil were distributed randomly throughout the phylogeny. Some of the isolates had identical sequence with FOL isolates. These results support our hypothesis.

GENE GENEALOGIES REVEAL POTENTIAL NEW SPECIES WITHIN THE *GIBBERELLA FUJIKUROI* SPECIES COMPLEX. M.M. Jimenez-Gasco¹, G.A. Kuldau¹, N.C. Zitomer², D.D. Archibald³, E.M. Snyder⁴ and D.M. Geiser¹. ¹*Department of Plant Pathology, The Pennsylvania State University, University Park, PA, USA.* ²*USDA-ARS, Toxicology and Mycotoxin Research Unit, Athens, GA, USA.* ³*Department of Crop and Soil Sciences, The Pennsylvania State University, University Park, PA, USA.* ⁴*Huck Institute of Life Sciences, The Pennsylvania State University, University Park, PA, USA. E-mail: jimenez-gasco@psu.edu*

The *Gibberella fujikuroi* species complex (GFC) is a diverse, biogeographically structured group of taxa that includes important plant pathogens and mycotoxin producers, particularly fumonisins. The species name *Fusarium moniliforme* has been applied for a long time to members of at least eleven biological species that correlate to mating populations, and to more than fifty phylogenetic species within the GFC. The elongation factor 1-alpha gene phylogeny of over 500 diverse isolates previously accessioned as *F. moniliforme* in the Fusarium Research Center (FRC) culture collection led to the identification of new potential species within the GFC. Most of these potential new species fall within the African clade of the GFC. Species boundaries of potential new taxa in the GFC, particularly targeting those within the African clade, were addressed through additional phylogenetic analyses using sequences from the beta-tubulin gene and intergenic spacer of the nuclear ribosomal RNA gene cluster. Multilocus phylogenies identified at least one cryptic species closely related to *F. andiyazi* with potential to produce fumonisins. Most members of this and other potential new species were associated to African crops, such as sorghum, millet and pearl millet, indicating that these crops are a source of species diversity within the GFC larger than initially expected. Analyses of mycotoxin production revealed that new *Fusarium* spp. associated with African

crops are capable of producing fumonisins *in vitro* which may pose a potential risk to human and animal grain consumption.

FUSARIUM ISOLATES REPRESENTING NOVEL LINEAGES IN THE *GIBBERELLA FUJIKUROI* COMPLEX COLONIZE MALFORMED *SYZYGIUM CORDATUM* INFLORESCENCES. M. Kvas^{1,3}, E.T. Steenkamp^{1,3}, B.D. Wingfield^{2,3}, W.F.O. Marasas³ and M.J. Wingfield^{1,3}. ¹*Department of Microbiology and Plant Pathology.* ²*Department of Genetics, Centre of Excellence in the Tree Health Biotechnology (CTHB).* ³*Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. E-mail: emma.steenkamp@fabi.up.ac.za*

The *Gibberella fujikuroi* complex (GFC) includes numerous economically important *Fusarium* species that are responsible for a range of plant diseases. One of these diseases, mango malformation, has been linked to at least four different *Fusarium* species in the GFC. Recently, symptoms resembling those of mango malformation were observed on the native South African tree, *Syzygium cordatum* (Myrtaceae). Panicles of the affected *S. cordatum* inflorescences are typically excessively branched and thickened with a large number of malformed and sterile flowers. The aim of this study was to isolate and identify the *Fusarium* species associated with malformed *S. cordatum* inflorescences. For this purpose, we used the DNA sequence comparisons for the genes encoding EF-1 α and β -tubulin. Comparison of the various EF-1 α sequences with those in the *Fusarium* identification database (<http://fusarium.cbio.psu.edu/>) revealed that many of the *Fusarium* isolates obtained from the affected inflorescences represent members of the sections *Arthrosporiella*, *Gibbosum* and *Elegans*, while some also formed part of the GFC. To identify the GFC isolates, phylogenetic analyses based on the combined sequence data for EF-1 α and β -tubulin were used. The results showed that malformed *S. cordatum* inflorescences are colonized by at least ten different *Fusarium* species in the GFC. Although some of these appear to be conspecific with or closely related to known species (e.g. *F. proliferatum*), the majority represent novel lineages in the GFC. These surprising findings, suggest that many novel taxa in the GFC await discovery. They are also likely to provide valuable insights into the evolution of this important group of fungi.

GENETIC CHARACTERISATION OF *FUSARIUM OXYSPORUM* FROM NATURAL SOILS IN AUSTRALIA. M.H. Laurence, B.A. Summerell, L.W. Burgess and E.C.Y. Liew. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, Mrs Macquaries Road, Sydney, Australia. E-mail: matthew.laurence@rbgsyd.nsw.gov.au*

Fusarium oxysporum is a ubiquitous fungal species complex that includes both non-pathogenic and pathogenic strains, the latter being responsible for disease in over one hundred cultivated plant species. The origin of many of these strains is poorly understood but recent studies on the cotton wilt pathogen, *F. oxysporum* f. sp. *vasinfectum* in Australia, indicate an indigenous origin from populations associated with native cotton relatives. Given the broad host range of *F. oxysporum* and its potential threat to agriculture, there is a pressing need to characterise the native species complex as research to date has focused on isolates from agricultural environments. These are unlikely to represent the natural underlying diversity of the species complex in Australia due to anthropogenic distribution of pathogens and selection pressures that favour clonality. We have addressed this imbalance by sampling isolates associated with native vegetation geographically isolated from cultivation throughout the continent. DNA fingerprints were

obtained using various genetic markers to indicate the extent and distribution of genetic diversity. In addition the phylogenetic position and lineage composition of the native soil population were investigated on the basis of DNA sequences of the β -tubulin, EF-1 α , NIR, CAL and mtSSU rDNA regions. The evolutionary potential of native *F. oxysporum* populations in Australia is discussed.

THE CAUSAL AGENT OF FUSARIOSES ON PINEAPPLE IS GENETICALLY DIVERSE IN BRAZIL. C.S. Lima¹, S.S. Costa¹, N.C. Crespo¹, J.A. Ventura² and L.H. Pfenning¹. ¹Departamento de Fitopatologia, Universidade Federal de Lavras, 37200000 Lavras, MG, Brazil. ²INCAPER, Rua Alfonso Sarlo 160, 29052010, Vitória, ES, Brazil. E-mail: ludwig@ufla.br

The genetic diversity of *Fusarium guttiforme* (syn. *F. subglutinans* f. sp. *ananas*), the etiologic agent of fusariosis on pineapple in Brazil, was assessed for *Fusarium* isolates obtained from diseased pineapple plants from various regions through VCG analysis, and sequencing and phylogenetic analysis of a portion of the *tef1* and *tub2* genes. Pathogenicity of the isolates was tested by inoculation on pineapple leaves and plants. In the control treatments leaves and plants were inoculated with sterile water and with an isolate of *F. subglutinans* from mango. The isolates analyzed grouped in several VCGs, containing one to few isolates. The phylogenetic analysis of *tef1* and *tub2* DNA sequences revealed a high genetic variability with three distinct clades in the *Gibberella fujikuroi* species complex. All clades of *F. guttiforme* formed one major clade, closely related to *F. begoniae*. On the other hand, none of the isolates from pineapple grouped in the *F. subglutinans sensu stricto* clade, confirming that pineapple fusaria represent at least one different species. Representative isolates were pathogenic to pineapple plants, which showed typical necrotic areas at the inoculation site, while control plants did not develop fusariosis symptoms after inoculation. Leaves inoculated with *F. guttiforme* isolates also produced necrotic lesions, while in the control they remained with no necrotic areas. The high number of VCGs and the sequence variability within the *F. guttiforme* population observed in the present study suggest a high genetic diversity in the population of *F. guttiforme* that causes the pineapple fusariosis in Brazil. Possible sexual reproduction within the population is investigated.

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A TWO-LOCUS DNA SEQUENCE DATABASE FOR IDENTIFYING HOST-SPECIFIC PATHOGENS AND PHYLOGENETIC DIVERSITY WITHIN THE *FUSARIUM OXYSPORUM* SPECIES COMPLEX. K. O'Donnell¹, P. Johnston², P. Crous³, C. Gueidan³, P. Colyer⁴, A. Glenn⁵, R. Riley⁵, N. Zitomer⁵, C. Waalwijk⁶, D. Geiser⁷, S. Kang⁷, J. Juba⁷, D.A. Sutton⁸, R. Ploetz⁹, M. Elliot¹⁰, H.C. Kistler¹¹, M. Davis¹², S. Sink¹ and B. Sarver^{1,13}. ¹NCAUR-ARS-USDA, Peoria, IL, USA. ²Landcare Research, Auckland, New Zealand. ³Centraalbureau voor Schimmelcultures, Fungal Biodiversity Center, Utrecht, The Netherlands. ⁴Louisiana State University Agricultural Center, Bossier City, LA, USA. ⁵USDA-ARS, Athens, GA, USA. ⁶Plant Research International, Wageningen University and Research, Wageningen, The Netherlands. ⁷Department of Plant Pathology, The Pennsylvania State University, University Park, PA, USA. ⁸Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA. ⁹University of Florida, Homestead, FL, USA. ¹⁰University of Florida, Ft. Lauderdale, FL, USA. ¹¹USDA-ARS, Cereal Disease Laboratory, St. Paul, MN, USA. ¹²Department

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An electronically portable two-locus DNA sequence database, comprising partial sequences of the translation elongation factor gene (*EF-1 α* 634 bp alignment) and nearly complete sequences of the nuclear ribosomal intergenic spacer region (IGS rDNA, 2220 bp alignment) for 850 isolates spanning the phylogenetic breadth of the *Fusarium oxysporum* species complex (FOSC), was constructed to investigate evolutionary relationships and provide discrete nucleotide sequence-based markers for identifying host-specific pathogens (i.e., *formae speciales*), putative non-pathogens, and opportunistic pathogens of humans and other animals within the FOSC. Of the 850 isolates subjected to the DNA-sequence based typing scheme, 101 *EF-1 α* , 204 IGS rDNA, and 257 two-locus sequence types (STs) were differentiated. One hundred and forty of the two locus STs were singletons. The human mycose-associated isolates were widely distributed throughout the FOSC phylogeny and represent at least 25 putatively clonal lineages. Several of these lineages are exclusively associated with mycotic infections, whereas others include isolates from hospital or industrial plumbing systems. Mycotoxin analyses of twenty isolates representing the phylogenetic breadth of the FOSC indicate that moniliformin (MON) and fumonisin (FUM) production may be very limited in that only one strain produced MON and one FUM. This data will be incorporated into the next version of the web-accessible *Fusarium-ID* database.

MOLECULAR PHYLOGENETIC DIVERSITY, MULTILOCUS HAPLOTYPE NOMENCLATURE, AND *IN VITRO* ANTIFUNGAL RESISTANCE WITHIN THE *FUSARIUM SOLANI* SPECIES COMPLEX. K. O'Donnell¹, D.A. Sutton², A. Fothergill², D. McCarthy², M.G. Rinaldi², M.E. Brandt³, N. Zhang⁴ and D.M. Geiser⁵. ¹NCAUR-ARS-USDA, Peoria, IL, USA. ²Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA. ³Centers for Disease Control and Prevention, Atlanta, GA, USA. ⁴Department of Plant Pathology, Cornell University, Geneva, NY, USA. ⁵Department of Plant Pathology, The Pennsylvania State University, University Park, PA, USA. E-mail: kerry.odonnell@ars.usda.gov

Members of the species-rich *Fusarium solani* species complex (FSSC) are responsible for approximately two-thirds all fusarioses of humans and other animals. In addition, many economically important phytopathogenic species are nested within this complex. Due to their increasing clinical relevance and because most of the human pathogenic and plant pathogenic FSSC lack Latin binomials, we have extended the multilocus haplotype nomenclature system introduced in a previous study (Chang *et al.*, *JAMA* 296: 953-963, 2006) to all 35 species within the medically important FSSC clade 3 to facilitate global epidemiological studies. The typing scheme is based on polymorphisms in portions of the following three genes: the internal transcribed spacer (ITS) region plus domains D1+D2 of the nuclear large subunit (LSU) ribosomal RNA, translation elongation factor (*EF-1 α*), and the second largest subunit of RNA polymerase II (*RPB2*). Of the 252 isolates subjected to multilocus DNA sequence typing, 192 sequence types were differentiated, and these were distributed among three strongly supported clades designated 1, 2 and 3. All of the mycose-associated isolates were restricted to FSSC clade 3, and these represent at least 21 phylogenetically distinct species. Analyses of the combined DNA sequence data, using 3 separate phylogenetic methods, yielded the most robust hypothesis of evolutionary relationships and genetic diversity within the FSSC to date. The *in vitro* activity of 10

antifungals tested against 20 isolates representing 18 species that span the breadth of the FSSC phylogeny show that members of this complex are broadly resistant to these drugs.

A NOVEL SPECIES WITHIN THE *FUSARIUM GRAMINEARUM* COMPLEX FROM ETHIOPIA DETECTED BY A MULTILOCUS GENOTYPING ASSAY AND MOLECULAR PHYLOGENETICS. K. O'Donnell¹, T.J. Ward¹, D. Abera², T. Aoki³, H.C. Kistler⁴, Å. Bjørnstad² and S.S. Klemsdal². ¹NCAUR-ARS-USDA, Peoria, IL, USA. ²Bioforsk, Norwegian Institute of Agricultural and Environmental Research, Høgskoleveien, Norway. ³Gene Bank-Microorganisms Section (MAFF), National Institute of Agrobiological Sciences (NIAS), 2-1-2 Kannondai, Tsukuba, Ibaraki, Japan. ⁴Cereal Disease Laboratory, ARS-USDA, St. Paul, MN, USA. E-mail: kerry.odonnell@ars.usda.gov

Twenty isolates resembling members of the *Fusarium graminearum* species complex (*Fg* complex; O'Donnell *et al.*, *Fungal Genet. Biol.* 41: 600-623, 2004) were isolated from ground wheat samples collected in two different geographic areas in Ethiopia. Results of a multilocus genotyping (MLGT) assay (Ward *et al.*, *Fungal Genet. Biol.* 2007, in press) suggested that these isolates might represent a new species within the *Fg* complex. To further assess species identity, phylogenetic analyses of multilocus DNA sequence data (13 genes; 16.3 kb/strain) resolved the Ethiopian isolates as a novel, strongly supported monophyletic sister group to *F. acaciae-mearnsii* within the *Fg* complex. These isolates also appear to be novel in that MLGT probes targeting *Tri3* or *Tri12* near the ends of the trichothecene (TRI) gene cluster suggested that the TRI cluster reflects recombination between isolates with a 15-acetyldeoxynivalenol (15ADON) and nivalenol (NIV) chemotype. Sequence analysis of either end of the TRI cluster identified 15ADON and NIV recombination blocks, demonstrating inter-chemotype recombination within the TRI cluster for the first time. Results of pathogenicity experiments and trichothecene analyses show that this novel *Fg* complex species can cause *Fusarium* head blight on wheat and elaborate 15ADON *in planta*.

GENETIC STRUCTURE OF *FUSARIUM THAPSINUM* ISOLATES FROM GRAIN SORGHUM IN AUSTRALIA. T. Petrovic¹, E.C.Y. Liew², L.W. Burgess³ and B.A. Summerell². ¹CSIRO Entomology, PMB 2, Glen Osmond, SA 5064, Australia. ²Botanic Gardens Trust, Royal Botanic Gardens and Domain, Mrs. Macquaries Road, Sydney, NSW 2000, Australia. ³Faculty of Agriculture, Food and Natural Resources, The University of Sydney, Sydney, NSW 2006, Australia. E-mail: Tijana.Petrovic@csiro.au

Fusarium thapsinum is the dominant species associated with grain sorghum in the northern grain belt of NSW, Australia. There have been no studies on the population genetic structure of this pathogen in the continent. Consequently, we characterise *F. thapsinum* populations through determination of the genetic structure at various spatial levels of comparison (plant part, plot and geographic location) in the northern grain belt of NSW. These comparisons will give an insight into the overall relatedness of *F. thapsinum* populations. In this study a total of 311 isolates were analysed, representing *F. thapsinum* populations at various spatial levels of comparison. High levels of genetic diversity were shown for this species. Sexual reproduction in all *F. thapsinum* populations was a relatively rare event, ranged from 125 to 249 asexual generations per sexual generation (plant part and plot populations) and 145 to 290 (geographic populations). However, sexual reproduction in combination with other factors,

might be frequent enough to generate the high levels in genetic diversity of populations. No genetic differentiation was observed among plant part populations due to extensive gene/genotype flow. Genetic differentiation among plot and geographic populations may indicate that some populations experienced a lag in gene/genotype flow. In practice, the knowledge gained in this study may allow the development of more targeted disease management programs for *Fusarium* stalk rot of grain sorghum.

FIRST REPORT OF A NOVEL *FUSARIUM* SPECIES CAUSING SUGAR BEET YELLOW. V. Rivera¹, M. Khan¹, J. Rengifo¹, D.M. Geiser² and G. Secor¹. ¹Department of Plant Pathology, North Dakota State University, Fargo, ND, USA. ²*Fusarium* Research Center, Department of Plant Pathology, Pennsylvania State University, University Park, PA, USA. E-mail: gary.secor@ndsu.edu

Fusarium yellow disease of sugar beet (*Beta vulgaris*) can cause serious field losses, due to wilt and early death, and can be only controlled by planting resistant cultivars. Yellow disease is caused primarily by *Fusarium oxysporum*, but other *Fusarium* species have been implicated. Severe outbreaks of yellows disease has been reported in southeast ND and southwest MN. Isolations were made from diseased sugar beets collected in the field or *Fusarium* screening nurseries with yellowing, interveinal chlorosis, necrosis, stunting and vascular discoloration of the tap root to identify causal agents. *F. oxysporum*, *F. graminearum*, and a new species of *Fusarium* were recovered from the plants. All *Fusarium* species can be isolated from tap roots, but only the new *Fusarium* species can be isolated from petioles. Pathogenicity of the new *Fusarium* was tested in greenhouse using a *Fusarium* yellow susceptible cultivar. All isolates were highly pathogenic, causing typical yellowing symptoms. On PDA medium produce a bright orange mycelia, macro and microconidia are present but sparse. Neosolanol and 4,15-diacetoxyscirpenol toxins were detected using Thin Layer Chromatography (TLC); RFLP-ITS characterization based on *AluI* and *Fnu4HI* digest of the ITS region shows the new *Fusarium* has a distinct restriction patterns. Preliminary data, based in the partial translation elongation factor-1 α (TEF) sequences, mycotoxin production assayed, and morphological description suggest a new *Fusarium* species, a possible member of the *Giberella fujikuroi* complex and related but not identical to *F. acutatum*, that had not previously been reported as the cause of sugar beet yellows.

MORPHOLOGICAL AND MOLECULAR VARIATION AMONG SPECIES OF THE *FUSARIUM DIMERUM* SPECIES GROUP. H.J. Schroers¹, S.C. Lamprecht², K. O'Donnell³, P.L. Kammeyer⁴, S. Johnson⁴, D.A. Sutton⁵, M.G. Rinaldi⁵ and R.C. Summerell⁶. ¹Agricultural Institute of Slovenia, Haquetova 17, Ljubljana, Slovenia. ²Agricultural Research Council-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa. ³NCAUR-ARS-USDA, Peoria, IL, USA. ⁴Loyola University Medical Center, Maywood, IL, USA. ⁵Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX, USA. ⁶Sporometrics Inc., 219 Dufferin Street, Suite 20C, Toronto, Ontario, M6K 1Y9, Canada. E-mail: bans.schroers@kis.si

The name *Fusarium dimerum* has been used in the past for saprotrophic fungi and opportunistic human pathogens with up to 3-septate but mostly 0- or 1-septate *Fusarium*-like conidia. On the basis of narrowly defined morphological characters, the varieties *Pusillum*, *Nectrioides* and *Violaceum* were distinguished but phylogenetic analysis on the basis of sequences of 4 loci indicate

that the morphospecies *F. dimerum* comprises more than 10 phylogenetic species. When formed on the agar surface, septate conidia are produced in these species only sparsely and swell and become vacuolated quickly. Therefore, they cannot be used for refined morphological characterizations. On surfaces of carnation leaf pieces placed on synthetic nutrient-poor agar, most species of the *F. dimerum* group form well-developed sporodochia with consistently shaped macroconidia and with a uniform number of septa, with the help of which some of the phylogenetic species can be morphologically segregated. Six of these species form predominantly 2-septate conidia or immature conidia, where the single septum is placed asymmetrically. By way of contrast, several other species form sporodochial conidia with a single, more or less medial septum. Among the species with 2-septate septa, one is characterized by almost annulate conidia, while conidia of the other species can either be more strongly curved distally than proximally or lunate. Similarly, conidia in species with 1-septate spores can either be almost straight but with bent and pointed ends or lunate. The morphological and molecular variation among the phylogenetic species of the *F. dimerum* species group so far recognized is illustrated.

TYPIIFICATION OF *FUSARIUM CEREALIS* AND *FUSARIUM CROOKWELLENSIS*. K.A. Seifert, T. Gräfenhan and S.A. Redhead. *Biodiversity (Mycology and Botany), Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada. E-mail: seifertk@agr.gc.ca*

Fusarium cerealis and *F. crookwellense* are names applied to the same species. The first name, which has nomenclatural priority, is more commonly used, but the second name is still frequently employed. Critical examination of the literature demonstrates that the presumed type of *F. cerealis* in K is not the holotype and we were unable to locate original material in any other herbarium. The epithet *cerealis* was used in *Fusarium* by Wollenweber for a variety of *F. culmorum* that seems to represent the modern concept of *F. cerealis*. The International Code of Botanical Nomenclature allows us to designate a neotype for *F. cerealis*. The only option for maintaining usage of the name *F. crookwellense* would be to propose its conservation, a strategy that would be unlikely to succeed because of the dominant usage of *cerealis*. Therefore, the most likely solution is to recommend abandonment of *F. crookwellense*, and to formalize the synonymy by neotypification of *F. cerealis* with a conspecific specimen.

A *FUSARIUM* POPULATION FROM BANANA MISSING THE FUMONISIN GENE CLUSTER: A NEW SPECIES CLOSELY RELATED TO *F. VERTICILLIOIDES*. F. Van Hove¹, C. Waalwijk², M.T. González-Jaén³, A. Moretti⁴ and F. Munaut¹. ¹*Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL#), Unité de Microbiologie, Faculté d'Ingénierie Biologique, Agronomique et Environnementale, Université Catholique de Louvain, Croix du Sud 3 bte 6, 1348 Louvain-la-Neuve, Belgium.* ²*Business unit Biointeractions and Plant Health, Plant Research International, Wageningen, The Netherlands.* ³*Department of Genetics, University Complutense of Madrid, Spain.* ⁴*Institute of Sciences of Food Production, National Research Council, Bari, Italy. E-mail: cees.waalwijk@wur.nl*

Several strains of *Fusarium* isolated from banana were initially identified as *F. verticillioides* and described as non fumonisin producing strains. Here, we report preliminary results indicating that a sequence of 44 kb including the main part of the Fumonisin Gene Cluster (FGC) has been excised from these *Fusarium* strains. The excision hypothesis is supported by the fact that the 5' end (756 bp) of the most proximal gene of the FGC (*FUM21* that regulates *FUM* gene expression) and the 3' end (902 bp) of the most distal gene of the FGC (*FUM19* that encodes an ABC transporter) are still present in the genome of these strains. This result explains previous unfruitful attempts to amplify by PCR or reveal by southern blot hybridisation, the presence of several genes of the FGC in, as well as the non production of fumonisins by these banana strains. Molecular data analyses on tEF, ITS, 28S, calmodulin, beta-tubulin and mtSSU sequences we report here show significant variations with sequences from species of the *Gibberella fujikuroi* complex including *F. verticillioides*. Together with previous results of molecular studies (AFLP fingerprinting and tEF, mtSSU and IGS sequences), as well as with results of crossing experiments, pathogenicity tests and morphological characterization these new data indicate that these strains isolated from banana represent a new *Fusarium* species, which is closely related to *F. verticillioides*.

POLYPHIALIDES ARE NOT A RELIABLE CHARACTER IN THE TAXONOMIC DISTINCTION OF *FUSARIUM VERTICILLIOIDES* AND *F. PROLIFERATUM*. I. Visentin, V. Montis, D. Valentino, G. Tamietti and F. Cardinale. *Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (DiVaPRA), Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco TO, Italy. E-mail: francesca.cardinale@unito.it*

The maize pathogens *F. verticillioides* and *F. proliferatum* are morphologically very similar; for this reason, they were grouped in one species (*F. moniliforme*) until a few years ago (Seifert *et al.*, *Mycol. Res. News* 107: 643, 2003). The only accepted marker for these morphospecies is the possible (but not exclusive) formation of polyphialides in *F. proliferatum* but not in *F. verticillioides*; both produce microconidia in chains. A collection of isolates from maize grown in North-western Italy were assigned either to *F. verticillioides* or *F. proliferatum* by morphological observation. This approach was integrated with sexual and molecular analyses, to set the biological and the phylogenetic species borders in our collection, and to compare them with those of the morphospecies. Given the discrepancies in the results of the three approaches, we suggest that the biological, morphological and phylogenetic borders of these species do not always strictly overlap as currently assumed. In particular, about 25% of the strains characterized as *F. verticillioides* by three independent phylogenetic analyses (AFLP, ITS and calmodulin sequencing) produce polyphialides, indicating that these species are not distinguishable at the morphological level. We also propose that the ITS region be considered taxonomically relevant for the phylogenetic distinction of these two species. Indeed ITS-RFLP, as well as a pair of primers designed on conserved polymorphisms associated to either species within this region, could rapidly and very reliably discriminate between *F. proliferatum* and *F. verticillioides* isolates, once they were assigned to the *ex*-species *F. moniliforme* by looking for the presence of conidial chains.