# Abstracts of the 32nd Meeting of the KNPV Fusarium working group

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## *Topographically triggered mycelial bundles in Acremonium and Fusarium species*

*Fusarium oxysporum. Image: Jan Dijksterhuis.*

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Culturing microorganisms in new ways can produce surprising changes in morphology. Micro-cultivation chips (MCC) are one such novel method that can support the cultivation of fungi as microcolonies. MCC comprise an array of microwells (from 20 to 300 microns diameter) with a porous ceramic base. A novel species of *Acremonium* and a number of *Fusarium* species (including *F. oxysporum*) produce mycelial bundles when cultured on MCC. These bundles maintain the diameter of the wells, despite growing out of the wells – they appear to be templated by the early confinement in the microwells. We are working to understand how these structures form, what they do and how they relate to the natural situation of these fungi.

## *Pathogenicity chromosomes as Trojan horses? The costs of mobile DNA in pathogen evolution.*

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Pathogenic *Fusarium oxysporum* (Fo) strains are host-specific and cluster genes involved in infection together with transposons on separate, dispensable pathogenicity chromosomes. Horizontal transfer of a pathogenicity chromosome can transform a non-pathogenic strain into a pathogenic one. Yet, for Fo pathogenicity is the exception rather than the rule. This suggests that the costs of obtaining a pathogenicity chromosome counterbalance the positive effects of being able to infect a new host. One example of such a cost is the fact that transposons that reside on a pathogenicity chromosome may colonize and disrupt the rest of the genome.

We are currently investigatinge the level and timescale of genome colonization by transposons from a transferred chromosome by reconstructing transferred chromosomes and subsequent transposon insertion events within several distinct Fo clonal lines. Moreover, we are applying experimental evolution using strains that obtained pathogenicity chromosomes in the lab, to study genome dynamics directly after horizontal transfer, *in vitro* and *in planta*. Together these two approaches will give insight into the dynamics of genome organization and the likelihood of emergence of new diseases through horizontal chromosome transfer.

## *Pathogenicity chromosomes in Fusarium oxysporum*

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Tomato-infecting strains *Fusarium oxysporum* f.sp. *lycopersici* (Fol) contain pathogenicity chromosomes. These chromosomes determine host range and can be transferred to a non-pathogenic strain, turning the recipient strain into a pathogen (Ma *et al*., 2010). Surprisingly, loss of a big part of the pathogenicity chromosome in a strain of Fol does not affect virulence (Vlaardingerbroek I, *et al*., 2016). To investigate which parts of the chromosome in Fol are required for pathogenicity to tomato plants and which parts can be transferred, we labeled several positions of the pathogenicity chromosome in Fol with different marker genes (encoding green fluorescence and red fluorescence). We then used fluorescence-assisted cell sorting (FACS) to select spores that have lost green fluorescence or red fluorescence and thus obtained several lines with a variety of deletions in this chromosome. We are currently testing virulence of these lines and aim to perform horizontal chromosome transfer experiments to assess whether these partial chromosomes can be transferred. Finally, we aim to characterize the pathogenicity chromosome of a strain that is specific to melon – *Fusarium oxysporum* f.sp. *melonis* (Fom) – and compare this to the previously identified pathogenicity chromosome of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (Forc), a strain that has a broad host range (cucurbits). We want to identify the regions or genes in Forc and Fom that are responsible for the difference in host range.

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## Targeting mtDNA improves quantification of Fusaria

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Quantitative polymerase chain reaction (qPCR) has been found to be the most promising alternative in quantification of fungi from food and feed. However, quantification of trace amounts of fungal DNA is often a challenge. This is mainly caused by relatively low fungal load in plant material. The sensitivity of detection of fungi can be improved by the use of diagnostic assays targeting multi-copy DNA regions, such as mitochondrial DNA. Recently, such an assay has been developed for quantification of *Fusarium graminearum* sensu stricto. The purpose of this study was to develop a highly sensitive mitochondrial based assay for quantification of *F. culmorum*. To ensure high specificity of the assay, primers and MGB probe (Minor-groove binding) were designed based on cox2 intron3, which is present in the mitogenome of *F. culmorum* only. Specificity of the assay was evaluated against 138 fungal strains including *F. culmorum* and other non-target fungal species. The assay was further evaluated for efficiency and sensitivity against different *F. culmorum* strains with various levels of pure fungal DNA as well as the presence of background wheat DNA. It was also shown that 0.01 pg of fungal template could be reliably quantified in the presence of background DNA. The assay was used to quantify *F. culmorum* DNA using 108 grain samples with different trichothecene levels. A significant positive correlation was found between fungal DNA quantity and the sum of trichothecenes. We used different qPCR assays to determine species identity of fungal field isolates obtained from different regions of Poland in 2017 and 2018. *F. graminearum* s.s. was identified as the predominating species associated with Fusarium Head Blight of wheat in Poland.

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## The unforeseen evolutionary history of Fusarium mitochondrial genomes

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Mitochondrial genomes are usually considered to be non-recombining; however, our findings in both *Fusarium oxysporum* and *F. graminearum* show that there is mitochondrial recombination within this group. The two groups have quite different lifestyles: *F. graminearum* has homothallic genome organization and has an active sexual cycle, while *F. oxysporum* has a heterothallic genome organization and has a putative parasexual cycle. The fact that these organisms with significantly different lifestyles both have mitochondrial recombination indicates that mitochondrial recombination may be a wide-spread phenomenon in *Fusarium*.

Earlier studies have already detected signs of putative interspecies recombination of the mitochondrial genomes in the *Fusarium fujikuroi* species complex (FFSC). Phylogenetic trees inferred from

different mitochondrial genes gave conflicting topologies. It has also been shown that there is at least a low level of interfertility between species of this group. In our earlier work, we have identified a new variant of the so-called large variable region of the mitochondrial genome. This new variant was found and described in *F. oxysporum*. In our current analysis, we have found this variant in the mitogenome of some of the FFSC members, in all three major clades (African, American and Asian). The distribution of this variant also enforces the fact the interspecies crosses have played an important role in the evolution of the FFSC. Furthermore, it also suggests that there has been genetic exchange between the members of the *F. fujikuroi* and *F. oxysporum* species complexes.

## *Novel biocontrol agents "Fungal Endophyte" for Fusarium graminearum biocontrol in maize*

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Fusarium Head Blight (FHB) is a devastating fungal disease which affects small grain cereals such as wheat and maize. Although FHB is caused by a species complex, *Fusarium graminearum* (Fg) is the most important member. Beside the economic losses due to the decrease in yield, the fungus has an impact on the quality due to the production of mycotoxins. Additionally, these mycotoxins represent a serious impact on human and animal health upon consumption of the contaminated cereals. Driven by the awareness that reduced tillage systems result in soil structure improvement, conservation tillage practices are often implemented leaving more stubble/straw residues on the field. This organic material can serve as the primary inoculum of Fg. Over the last decade, different strategies for FHB management have been proposed. Biological control using beneficial or non-pathogenic bacteria and fungi is encouraged as it is a safe and sustainable long-term solution in comparison with chemical control. Although crop residues serve as primary inoculum of Fg, we hypothesize that these crop residues also harbor valuable antagonistic fungi which might be used as biocontrol agents. In the current project, several novel fungal endophytes have been isolated, from European and African crop residues, and tested for their ability to control the growth of Fg and the production of its mycotoxins *in vivo* and *in vitro*.
New isolates of *Sordaria* spp., *Clonostachys* spp., *Epicoccum* spp., and others were tested for their effects against Fg. *In vitro* plating assays (contact and volatile) and *in vivo*, maize pot experiments, have been performed for each isolated species to assess their biocontrol capacity against Fg. The obtained results indicate that the selected biocontrol agents have a promising effect on Fg infection. Furthermore, the selected biocontrol agents have an inhibitory effect on levels of mycotoxins (deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and zearalenone) measured through a validated multi-mycotoxin LC-MS/MS method production. Using a non-targeted approach, with Q-TOF LC/MS, the mechanism of action is being investigated: whether there is a detoxification effect and/or inhibitory volatiles or other substances that may have an effect on the fungus metabolism. The project will contribute to a great extend to reduction of *fusarium* mycotoxins level in grain cereals especially wheat and maize.

**The project is a part of the MYCOKEY project that aims at 'Integrated and innovative key actions for mycotoxin management in the food and feed chain'. The project is funded by Horizon 2020.**

## Research on the metabolism and renal excretion of deoxynivalenol in humans

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Deoxynivalenol is a highly common mycotoxin in cereals and cereals products and as a results it is one of the major mycotoxins in our diets. Thus, exposition studies showed the large exposure of human to this toxin with high percentages of population exceeding the tolerable daily intake. To know the exposition, analysis of urinary levels of deoxynivalenol and his glucuronides conjugates, which are the main phase II metabolites of deoxynivalenol, have been proposed as reliable biomarkers due to his short excretion half-life. So, the analysis of glucuronides forms in urine is crucial for the study of trichothecenes biomarkers, because about 90 % of deoxynivalenol excreted via urine is conjugated with glucuronic acid. For the glucuronides determination, a preliminary approach was developed based on the breakage of deoxynivalenol-glucuronides and subsequent determination of 'total deoxynivalenol' (sum of free and released mycotoxins by hydrolysis). Afterwards, a direct method for quantification of glucuronides such as deoxynivalenol-3-glucuronide and deoxynivalenol-15-glucuronide, which are the more common glucuronides forms, was developed. Later, strong correlations between the sum of urinary deoxynivalenol and its glucuronidated metabolites have been found and applied in several studies. These investigations revealed the power of biomarker driven work when compared to traditional exposure assessment by analyzing food stuff. Notwithstanding, some uncertainties are still present in the excretion metabolism and renal excretion of deoxynivalenol in humans. On one hand, there is a lack of information in the absorption and excretion rate of it. On the other hand, the high presence of deoxynivalenol conjugates in food like deoxynivalenol-3-glucoside or acetyl-deoxynivalenol add more uncertainties for the correlation between urinary deoxynivalenol and deoxynivalenol intake. Because of that, 20 volunteers (55 % women and 45 % men) were submitted an intervention diet without consuming cereals or cereals products for three days. Then, a tolerable daily intake (TDI) of deoxynivalenol (1 µg/kg body weight/day) were administrated to the volunteers who collected the urine for the next 24 hours. The aims of this study were the description of the deoxynivalenol and metabolites excretions patterns and know the absorption and excretion rates of it. Moreover, the results were useful to build an standardized method to estimate deoxynivalenol intake by means of biomarkers.

## The Fusarium/Neocosmospora paradox including new and interesting taxa from diverse substrates

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The taxonomic history of Fusaria has been marked by difficulties and controversies. Currently, the generic delimitation of *Fusarium* and related genera is still unsettled. Two opposite views are in use, however, both perspectives being nomenclaturally valid. Although a proposal has been presented to conserve the 'long standing use of the name *Fusarium*', particularly to retain the use of the name *Fusarium* for the *F. solani* species complex (SC) and other associated clades, this is not supported by currently available molecular, ecological and phenotypic data. A brief explanation of the available evidence is shown here in order to further explain the current delimitation of *Fusarium*, now confined to species producing *Gibberella* sexual morphs and the transfer of members of the *Fusarium solani* SC to the genus *Neocosmospora*. In addition, studies of freshly collected Fusaria from symptomatic *Citrus* spp. and diverse ornamental plants in the Mediterranean basin revealed the presence of six interesting new taxa: in *Fusarium* sensu stricto, a new linage is described containing two prominent citrus canker pathogens (*F. citricola* and *F. salinense*, in the *F. citricola* SC); *F. siculi* is described in the *F. fujikuroi* SC, associated with citrus dry-root rot symptoms. *Neocosmopora croci* and *N. macrospor*a are also described from citrus dry-root rot, while *Neocosmospora persicina* is described as a canker pathogen in *Persea americana*.

## Insights in the epidemiology and diversity of Fusarium oxysporum f. sp. cubense, the causal agent of Panama disease in banana

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Panama disease or Fusarium wilt of banana draws global attention. The currently developing epidemic of the so-called Tropical Race 4 (TR4) is caused by a single clone represented by vegetative compatibility group 01213. It is reminiscent of the previous epidemic that wiped out 'Gros Michel' bananas in Central America, which pushed the banana industry into bankruptcy. The epidemic was eventually quenched by cultivating 'Cavendish' bananas, which are resistant to the so-called Race 1 strains that caused the epidemic in 'Gros Michel'. The industry revived and thrives by the success of 'Cavendish' that has developed into a global monoculture. The emergence of TR4 wipes out 'Cavendish' plantations in South East Asia, where the disease spreads along with banana plantations expansions and from where it has spread into the Near and Middle East and Africa. Banana production in many regions is at stake and there are no sustainable solutions available. Our research focuses on the international complexity and addresses mostly genetic diversity in host and pathogen as well as epidemiological aspects embedded in multidisciplinary programs. We have used genotyping by sequencing technologies to describe global and regional diversity in the causal agent *Fusarium oxysporum* f.sp. *cubense* (Foc) and have phenotyped hundreds of banana accessions with various Foc genotypes. Methods to rapidly detect - particularly TR4 - and manage the disease have been developed to slow down the epidemic. This provides the necessary time for developing durable solutions that also contribute to break the hegemony of the global 'Cavendish' monoculture by introducing a diversified panel of banana cultivars. The latest developments will be presented and discussed.

## Panama disease of banana: learning from the Fusarium oxysporum-tomato interaction

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Panama disease, a wilt disease on banana caused by the fungus *Fusarium oxysporum f. sp. cubense* (Foc), has spread globally and severely affects banana plantations worldwide (Ploetz *et al.*, 2015; Ordonez *et al.*, 2015). The fungus is a soil inhabitant, reproduces asexually, and can reside in the soil very long periods in the form of chlamydospores. Tropical Race 4 (TR4) is one group of strains with high economic impact because it is able to infect all the commercial banana varieties. Attempts have been made to find ways to manage the disease, from ecological engineering to creating a pathogen-free cultivars (Ploetz, 2015; Thangavelu & Mustaff, 2012). However, none of them have yet been proved to be successful in controlling the disease.

In the *Fusarium oxysporum f.sp. lycopersici* (Fol) – tomato pathosystem the interaction between the fungal avirulence protein Avr3 and the I-3 immune receptor of tomato leads to disease resistance (Rep *et al.*, 2004; Van der Does *et al.*, 2088; Catanzariti *et al.*, 2015). Avr3 – also called Six1 – is a protein secreted in the xylem by Fol during infection. I-3 is naturally found in the tomato relative *Solanum pennellii*.

We asked the question whether a resistance gene of tomato could confer resistance in banana to Panama disease. We therefore investigated whether homologs of Six1 in Foc could interact with I-3 in the Fol-tomato pathosystem. Foc has three homologs of the *SIX1* gene designated as *SIX1a/b/c*. Our approach was to introduce these homologs into a Fol*ΔSIX1* mutant. As control we also reintroduced *SIX1* of Fol. All transformants were then tested on resistant tomato plants carrying the *I-3* gene. In parallel to that, transformants were also tested on susceptible tomato plants.

Our results show that the I-3 resistant plants infected by Fol *SIX1*::*SIX1a/b/c* have a lower disease index and a higher weight compared to the plants infected with *Fol ΔSIX1*. This suggests that *SIX1a/b/c* may interact with the *I-3* resistance gene, and induce an immune response. However, we also saw that *Fol SIX1::SIX1a/b/c* strains have reduced pathogenicity compared to the Fol wild type as well as *Fol SIX1::FolSIX1* strain. This suggests that Six1 homologs from Foc, while able to trigger I-3, also reduce pathogenicity of Fol on tomato.

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# *Fusarium oxysporum f.sp. lactucae, a major threat to lettuce production in Belgium*

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In Belgium, lettuce is mainly produced in soil in glasshouses with up to 5 crops per year. In 2015 the production of lettuce generated a revenue of almost 25 million Euro, with a production of 42,800 tonnes on 175 ha. During autumn 2015, wilting symptoms on butterhead lettuce were observed in two different commercial glasshouses in the Province of Antwerp. *Fusarium oxysporum* f.sp. *lactucae* was characterized as the causal agent of these symptoms. At this moment, already 13% of the glasshouse lettuce production area in Flanders (northern part of Belgium) is infested. Several physical, biological and chemical control measures were examined in a project called FUNSLA funded by VLAIO (Agentschap Innoveren en Ondernemen). The effect of steaming on *Fusarium oxysporum* propagules in the soil was investigated. Four hours of steaming resulted in a temperature of 50°C during 7 hours at 25 cm depth. Respectively 99% and 87% of *Fusarium* propagules were killed in the 0-20 cm and 20-40 cm soil layer. Next to that, different biocontrol organisms (*Bacillus subtilis*, *Gliocladium catenulatum*, *Trichoderma* etc.) chemical fungicides and fertilizers (such as Ortiva, Signum etc.) were tested in a pot experiment with naturally infested soil at a growers' glasshouse. Some biocontrol organisms had a positive effect on root quality, but had no statistically significant effect on symptom development in comparison with the infested control. Only the chemical product Ortiva gave slightly better results based on symptoms. Subsequently, different alternative lettuce types were tested in infested soil under lab and field conditions. Some Lollo bionda, Lollo rossa and oakleaf lettuce cultivars show intermediate resistance to *F. oxysporum* f. sp. *lactucae* and could form a good alternative. In the field no wilting symptoms were observed on lamb's lettuce, although on lab scale wilting was clearly noted. To conclude, at this moment the only solution for lettuce growers confronted with *F. oxysporum* f. sp. *lactucae* is to grow alternative lettuce types. The use of biological and chemical control needs further investigation.

# The presence of Fusarium species in Dutch onion fields

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Uireka is a research project, that aims to improve the quality and thereby strengthen the export position of the Dutch onion. To realize this, forces are bundled from the whole production chain. Innovations mainly concern renewal and improvement of cultivation and drying techniques. The quality of the onion directly after harvest and just before storage determines the quality further in the chain.

The Dutch Onion is a world player. Export is around 1.3 million tons of onions per year to approximately 130 countries world-wide. These are seed onions, planting onions and shallots. This means a value of more than 400 million euros. The export and hence the export value has grown 30 to 40% in the past 15 years. This position is due to an efficient chain structure and innovations by breeders, growers, suppliers, knowledge institutions, sorting and packing stations and exporters. In order to maintain or even expand that position, the quality must be excellent. In addition, it is necessary that the Dutch onion distinguishes itself as a durable product.

Part of Uireka deals with *Fusarium* research. In 2017, the focus is on the identification of *Fusarium* species in Dutch onion cultivation areas. From these areas, cultivation conditions are described and compared and onion bulbs are collected and examined for *Fusarium* infection. In total, bulbs are collected from 100 growers shortly before or after harvest. Participating growers will receive results of the survey on their parcel in the autumn.

During the presentation for the Fusarium Working Group, people will be informed about the project. Results will be presented in 2018. For more information consult the website http://uireka.nl/.


*Visual summary of subjects by working group secretary Anne van Diepeningen.*