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On the 31st of October 2018 the 33rd meeting of the KNPV working group Fusarium was held in Utrecht, at the Westerdijk Fungal Biodiversity Institute. An international community with representatives from three continents discussed to a variety of topics on *Fusarium***.** The first session focused on '*Fusarium oxysporum´*, a complex of species that is now being separated into multiple species. The genome of *F. oxysporum* is organized in a core component with especially the basic genes for being *Fusarium* and an accessory component containing most of the virulence information linking it to specific hosts. Transfer and recombination of accessory chromosomes may result in transition from a harmless, potentially beneficial endophyte to a pathogen. In the second session presentations were given of crown rot pathogens in wheat growing areas in China, the threesome interaction between *Fusarium culmorum*, bacteria in the soil microbiome and the plant host and on how stress in the plant may be used as a first identification for fungal and other infections. In the last session more details were disclosed on how *Fusarium oxysporum* may evolve into new pathotypes and on the fact that a fragment of an accessory chromosome is already sufficient to transfer virulence. The final speaker introduced us to the possibilities and limitations of genome editing based on the CRISPR-CAS technology in *F. graminearum* and *F. pseudograminearum*.

Abstracts of presentations of the 2018 Fusarium Day

Unraveling the *Fusarium oxysporum* enigma

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Fusarium oxysporum is the most economically important and commonly encountered species of *Fusarium*. This soil-borne fungus is known to harbour both pathogenic (plant, animal and human) and non-pathogenic strains. However, in its current concept *F. oxysporum* is a species complex consisting of numerous cryptic species. Identification and naming these cryptic species are complicated by multiple subspecific classification systems and the lack of living ex-type material to serve as basic reference point for phylogenetic inference. Therefore, to advance and stabilise the taxonomic position of *F. oxysporum* as a species and allow naming of the multiple cryptic species recognised in this species complex, an epitype is designated for *F. oxysporum*. Using multi-locus phylogenetic inference and subtle morphological differences with the newly established epitype of *F. oxysporum* as reference point, cryptic taxa can be resolved and described as species.

Return of the mitochondrial DNA

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Mitochondrial DNA sequences have been mostly neglected since the barcoding of life project started, due to technical difficulties in amplifying target regions. Because of this our knowledge of mitochondrial genomes and their evolution lags behind our understanding of the nuclear genome and its evolution. The combination of next generation sequencing and new assembly software tools make the efficient assembly of complete mitochondrial genomes possible. We used complete mitochondrial genomes and several nuclear markers to investigate the phylogeny of *Fusarium* sp., and to assess the value & potential of using mitochondrial genomics.

Based on the genealogical concordance phylogenetic species recognition method, the *Fusarium oxysporum* species complex (FOSC) consists of at least 3 phylogenetic species. Mitochondrial length variation and intron patterns support the separation of three phylogenetic species. The variable region of the mitogenome that is typical for the genus *Fusarium* shows two new variants in the FOSC. Comparative analysis of the mitogenomes in the FOSC revealed ongoing mitochondrial recombination within, but not between phylogenetic species. Besides FOSC isolates, some species from the *Fusarium fujikuroi* species complex (FFSC) contain one of the new variants of the large variable region, although the majority has the typical variant. Based on comparative phylogenetic analysis, the new variant has spread within the FFSC through horizontal gene transfer having its putative origin in the FOSC. This horizontal transfer was most probably mediated by introgression.

In conclusion, mitochondrial genomes can be efficiently assembled from next generation sequencing reads. Complete mitochondrial genomes offer a strong basis for phylogenetic studies. Furthermore, they may offer

new insights into the evolutionary history of the analyzed group, in our example the horizontal transfer between FFSC and FOSC species.

Finding marker genes for *formae speciales* of *Fusarium oxysporum* based on comparative genomics

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Fusarium oxysporum (*Fo*) is a soil-borne pathogen that can infect many plants. The species is divided into *formae speciales* (*ff.spp.*), based on host specificity; a single isolate can only infect one or a few closely related host plants. For example: *Fo f.sp. lycopersici* infects tomato plants, while *Fo f.sp. asparagus* infects asparagus plants. Besides plant pathogenic strains, there are also many non-pathogenic *Fo* strains. To be able to infect a certain host, an isolate requires a certain set of effectors – small-secreted proteins that are able to manipulate the host to establish a successful infection. To prevent spread of pathogenic *Fo* via contaminated seed or soil, we develop Taqman assays specific for a single *forma specialis* infecting either a vegetable or an ornamental crop. As target genes for these assays we use candidate effector genes identified using comparative genomics.

Population Analysis of Pathogen of Fusarium Crown Rot in China

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Abstract: Crown rot is a major constraint to dryland cereal production worldwide, many *Fusarium* species were reported to cause this disease, such as *F. culmorum*, *F. pseudograminearum*, *F. graminearum*, *F. avenaceum* and so on. In recent years, Fusarium crown rot of wheat outbreak much more frequently in China, however, the composition and variation of the pathogen are still largely unknown. In this study, we did a large-scale investigation of FCR in 12 provinces. A total of 3641 stains was isolated from 230 sampling sites. In Northern China, eighteen *Fusarium* species were identified. *F. pseudograminearum* (77%) was predominant species followed by *F. graminearum* (7.6%) and FIESC (2.4%). Fusarium composition varied in different regions, it seems associated with altitude, different from low altitude area, *F. culmorum* and *F. graminearum* were the main species of Inner Mongolia and Xijiang where altitude is higher than 800 meters. Significant higher ratio of *F. graminearum* (20%) was observed in east provinces Hebei and Shandong and in central province the frequency of *F. pseudograminearum* is higher than 90%. In Southern China, the composition is much more complicate. *F. asiaticum* and *F. graminearum* are the predominant species and ratio is just 39.8% and 29.4%Population analysis was performed on 264 representative *F. pseudograminearum* isolates by eight SSR makers. Only 88 haplotypes of 264 were identified and significant linkage disequilibrium ($p < 0.001$) was observed in all provinces, this indicated limited genetic exchange occurred within population and asexual reproduction is predominant in their life cycle. This study will be helpful for management of FCR in China.

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Observations of photosynthesis activity in tomato plants infected with *Fusarium solani*

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Since a few years Dutch tomato growers face more and more disease problems caused by *Fusarium solani*. For this reason *F. solani* was selected as one of the most relevant diseases to be studied in the project Healthy Plant Fundamentals. One of the objectives is to quantify how much faster pests and diseases can be detected in tomato before symptoms are visible with the naked eye. The basic idea behind the approach used for the early detection of diseases in this project is that pictures of chlorophyll fluorescence (Fv/Fm) are taken which give information about the level of photosynthesis activity which in turn is negatively correlated with the level of stress in the plant. Identified stressed plant parts can then be further analysed for the presence of a disease. Since the chlorophyll fluorescence (CF) camera can observe stress caused by disease long before the plant shows symptoms, this approach enables the detection of a disease before disease symptoms are visible for the naked eye, as has been shown before for instance for *Botrytis* in tomato. We discuss the results of a first test to observe photosynthesis activity in tomato plants (*cv.* Moneymaker) roots inoculated with *F. solani* and plan follow up experiments to study how the soil fungus *F. solani* can be non-destructively detected in above ground plant parts.
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Deciphering the genome and secondary metabolome of the plant pathogen *Fusarium culmorum* and its interactions with rhizobacteria

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Fusarium culmorum is one of the most important fungal plant pathogens that causes diseases on a wide diversity of cereal and non-cereal crops. The fungal strain *F. culmorum* PV was isolated from a sandy dune soil in the Netherlands. We observed that *F. culmorum* produced a unique cluster of volatiles, consisting primarily of terpenes. When exposed to the volatiles emitted by this fungus, the rhizobacterium *Serratia plymuthica* PRI-2C responded with an induction of motility and the production of the unusual terpene sodorifen. The sequence of the *F. culmorum* PV genome, revealed the presence of two terpene synthases, trichodiene- and longiborneol-synthase, which generate an array of volatile terpenes. Furthermore, we identified two gene clusters, deoxynivalenol and zearalenone, which encode for the production of mycotoxins. Linking the production of mycotoxins with *in vitro* bioassays, we found high virulence of *F. culmorum* PV on maize, barley and wheat.

Bad fungi gone good: control of *Fusarium* wilt disease with *Fusarium* endophytes

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Fusarium oxysporum (*Fo*) is known to cause vascular wilt disease in over 100 different hosts. Although most studies focus on its ability to cause disease, *Fusarium* is also capable of colonizing plants without triggering disease symptoms. Among these non-pathogenic strains, the endophytic strain *Fo*47 strain has repetitively been shown to confer protection against wilt disease on tomato caused by *Fo* f.sp. *lycopersici* (*Fol*).
We have acquired a collection of 80 non-pathogenic *Fo* strains isolated from non-cultivated soil or asymptomatic plants in three different continents (America, Australia and Europe) in order to assess how widespread is the capacity of non-pathogenic *Fo* to suppress *Fusarium* wilt disease. Bioassays with a subset of these non-pathogenic strains show that all strains tested have the ability to protect against *Fusarium* wilt

disease in tomato in 1:1 co-inoculation assays (endophyte: pathogen). Heat killed spores of the biocontrol strain are unable to trigger disease suppression showing that a living endophytic strain is needed to trigger disease suppression. Moreover, we found that pathogen abundance is reduced in the presence of *Fo*47 in root and stem tissue. Surprisingly, the colonization of *Fo*47 is increased in tomato stems when co-inoculated with *Fol*. Understanding the mechanisms behind disease suppression may help us to increase compatibility of plants with *Fo* endophytic strains and resistance to *Fusarium* wilt.

A partial pathogenicity chromosome in *Fusarium oxysporum* is sufficient to cause disease and can be horizontally transferred

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Pathogens secrete effectors to facilitate colonization of their hosts. In the case of *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*), fourteen effectors were identified in the xylem sap of infected tomato plants. So far, three effectors, including Six1, Six3, and Six5 have been proven to contribute to virulence. It turns out that all effector genes of *Fol* are located on the same chromosome, called the pathogenicity chromosome, which is required for virulence. Surprisingly, *Fol* strains missing a large part of the pathogenicity chromosome are still pathogenic. The *Fol* pathogenicity chromosome can also be horizontally transferred to a non-pathogen, turning the recipient strain into a pathogen. In order to assess which parts of the *Fol* pathogenicity chromosome are essential for infection, we selected different partial pathogenicity chromosome deletion strains. We first created a strain with the *RFP* gene on the short arm (p arm) of the pathogenicity chromosome and the *GFP* gene on the long arm (q arm). We then used fluorescence-assisted cell sorting to select *RFP*-only and *RFP*-only strains. By testing the virulence of these deletion strains, we show that less than 40% of the chromosome is sufficient for causing disease. In addition, we demonstrate that smaller versions of the pathogenicity chromosome can also be transferred and turn a non-pathogen into a pathogen.

CRISPR-Cas genome editing in *Fusarium poae*

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CRISPR-Cas technology is an efficient genome editing tool, which has been successfully used in several fungal genera such as *Penicillium*, *Aspergillus* and *Neurospora*. Yet, the general implementation of CRISPR-Cas in fungal genome editing to tackle biological questions remains limited. Our work aims at establishing a CRISPR-Cas9 platform in *Fusarium poae*. This pathogen is, despite its low virulence, omnipresent in the Fusarium head blight disease complex in Europe. The CRISPR-Cas9 tool is a versatile genome-editing tool which will allow us to unravel the (endophytic) life style of *F. poae*.
We used a dual expression system of Cas9 and sgRNA. First, we codon-optimized the Cas9 coding sequence for *F. poae* (opti-Cas9). This codon-optimized Cas9 was equipped with a tef1 promoter. The sgRNA fragment was put under the control of a U6 *F. poae* promoter (PpU6) or a U6 *F. graminearum* promoter (PgU6). We selected URA5 as target gene, as it has only one copy in the genome of *F. poae* and *F. graminearum*, and URA5 mutant could be positively selected in medium containing 5-FOA and exogenous uracil and negatively selected in medium without exogenous uracil.
After the transformation, we obtained potentially positive transformants which grew on FOA and uracil plus medium but did not grow on MEA medium without Uracil. These results point to the URA5 gene being knocked-out. We are currently Illumina Sequencing the genome of CRISPR mutants and wild type to confirm the mutant phenotype.