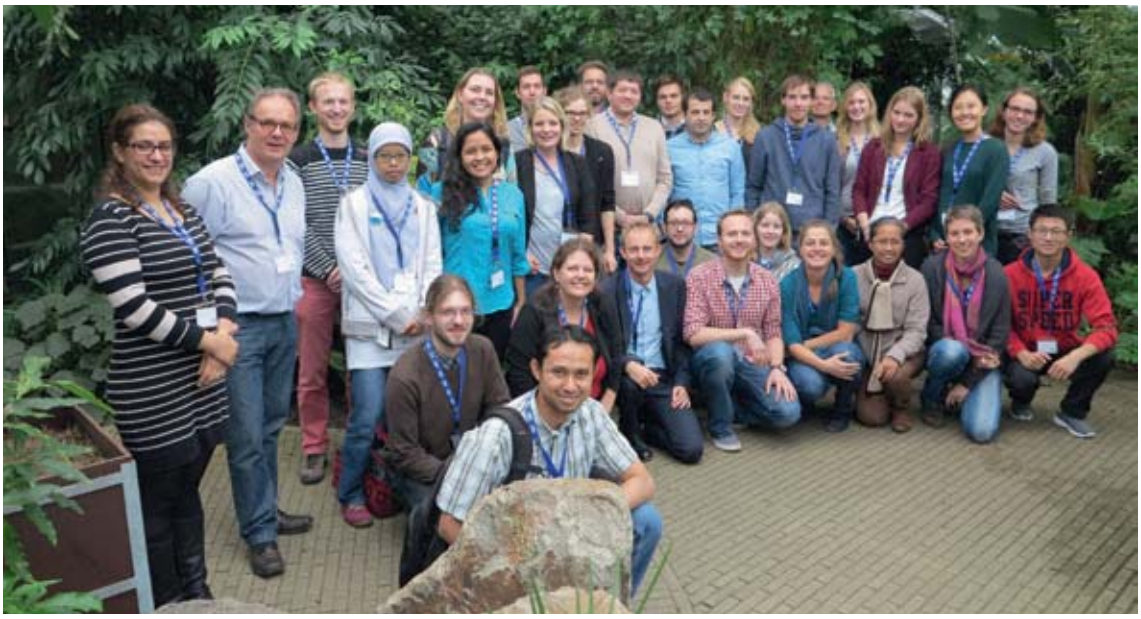
Werkgroep Fusarium

Samenvattingen van de presentaties gehouden tijdens de 30e bijeenkomst van de werkgroep.

Op woensdag 28 oktober kwam de werkgroep voor de 30e keer bijeen, ditmaal bij het Centraalbureau voor de Schimmelcultures in Utrecht. De groep aanwezigen was zeer internationaal van aard en hoorde acht wetenschappelijke presentaties. De onderwerpen varieerden van mycotoxines, via de *Fusarium*-epidemie in banaan tot de genoomstructuur van *Fusarium*. De Engelstalige samenvattingen zijn hieronder bijgevoegd.

Tijdens de vergadering is er een wisseling geweest in het bestuur. Na tien jaar is Martijn Rep (UvA) teruggetreden als secretaris van de werkgroep. De leden danken hem hartelijk voor zijn inzet. Hij wordt opgevolgd door Anne van Diepeningen (CBS-KNAW; a.diepeningen@cbs.knaw.nl). De volgende bijeenkomst van de werkgroep is vastgesteld op 26 oktober 2016, wederom bij het CBS.



Groepsfoto van de aanwezigen tijdens het lunchbezoek aan de botanische tuin in Utrecht.

Predictive Modelling of Mycotoxins in Cereals

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Predictions of deoxynivalenol (DON) content in wheat at harvest can be useful for decision-making by stakeholders of the wheat feed and food supply chain. In 2012, a forecasting model for DON in wheat cultivated in the Netherlands has been published (Van der Fels-Klerx *et al.* 2012) and later this model has been validated (Van der Fels-Klerx, 2014). The objective of the current research was to further improve quantitative predictions for DON in mature winter wheat in the Netherlands and to make them accessible for two specific groups of end-users. One model was developed for use by farmers in underpinning *Fusarium* spp. disease management, specifically the application of fungicides around wheat flowering (Farmer model). The second model was developed for industry and food safety authorities, and considered the entire wheat cultivation period (collector model). Model development was based on observational data collected from 638 fields throughout the Netherlands between 2001 and 2014. For each field, agronomic information, climatic data and DON levels in mature wheat were collected. Using multiple regression analyses, the set of biological relevant variables that provided the highest statistical performance was selected. Model validation showed good correlation between the predicted and observed DON levels. The two models maybe applied by various groups of end-users to reduce DON contamination in wheat-derived feed and food products and, ultimately, reduce animal ad consumer health risks. Future research will also focus on mechanistic models to mimic *Fusarium* ssp. life cycle and toxin production.

Microbial degradation of DON

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Contamination of food and feed by mycotoxins poses major risks for human or animal health and leads to economic losses. Next to prevention and intervention measures on the field, remediation of mycotoxins in contaminated feed is a growing sector. Remediation is already applied through the use of binders (clay or yeast derived products), but this adsorption between the binder and mycotoxin is reversible and pH depending. Moreover, these binders negatively influence the transfer of medication to the bloodstream. Therefore, there is need to develop other detoxification strategies. This can be achieved through microbial degradation of mycotoxins. Matrices with potential levels of mycotoxins or other complex molecules will be screened on the presence of microorganisms which can break down mycotoxins. The focus will be on mycotoxins DON and enniatin B. The incidence of these mycotoxins is respectively 100% and 91% in Flemish corn silage . Enrichment cultures for DON at 50 mg/kg have been established for the matrices: sheep rumen fluid, monoculture corn field soil, digestate from an anaerobic digester plant and activated sludge from a water treatment plant. Clear detoxification of DON by the enrichment cultures of soil and activated sludge was assessed with a bio-assay using *Lemna minor* (duckweed), whereas the actual degradation of DON through these cultures was confirmed with ELISA.

Exposure to green leaf volatiles primes wheat against FHB but boosts production of the mycotoxine DON

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Priming refers to a mechanism whereby plants are sensitized to respond faster and/or more strongly to future pathogen attack. Here, we demonstrate that pre-exposure to the green leaf volatile (GLV) Z-3-hexenyl acetate (Z-3-HAC) primed wheat (*Triticum aestivum* L.) for enhanced defense against subsequent infection with the hemibiotrophic fungus *Fusarium graminearum*.
Bioassays showed that after priming with Z-3-HAC wheat ears accumulated up to 40% lower necrotic spikelets. Furthermore, leaves of seedlings showed significantly smaller necrotic lesions compared to nonprimed plants, coinciding with strongly reduced fungal growth *in planta*. Additionally, we found that *F. graminearum* produced more deoxynivalenol, a mycotoxin, in the primed treatment. Expression analysis of salicylic acid (SA) and jasmonic acid (JA) biosynthesis genes and exogenous MeSA and MeJA applications showed that plant defense against *F. graminearum* is sequentially regulated by SA and JA during the early and later stages of infection, respectively. Interestingly, analysis of the effect of Z-3-HAC pre-treatment on SA and JA-responsive gene expression in hormone-treated and pathogen-inoculated seedlings revealed that Z-3-HAC boosts JA-dependent defenses during the necrotrophic infection stage of F. graminearum but suppresses SA-regulated defense during its biotrophic phase. Furthermore, we found an increase in the production of the plant hormones giberellic acid and indol acetic acid in the primed treatment. These possible roles of these plant hormones will also be discussed. Together these findings highlight the importance of temporally separated hormone changes in molding plant health and disease and support a scenario whereby the GLV Z-3-HAC protects wheat against *Fusarium* head blight by priming for enhanced JA-dependent defenses during necrotrophic stages of infection.

Worse comes to worst: Bananas and Panama disease - when plant and pathogen clones meet.

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Despite rich genetic and phenotypic diversity present in banana (*Musa*) species, only few cultivars developed over time into global commodities such as the triploid 'Cavendish' clones. In addition to this, tissue culture techniques facilitated the rapid rollout of these genetically identical banana plants onto vast acreages around the world. Thus, banana monoculture plantations are left vulnerable to diseases, such as Fusarium wilt and Black Sigatoka, given their clonal feature. *Fusarium oxysporum* f.sp. *cubense* (Foc) is the causal agent of Fusarium wilt or Panama disease

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that is again threating banana production around the globe. In the first half of the 20th century, Foc race 1 strains wiped out most of the 'Gros Michel' banana plantations in Latin America. 'Gros Michel' was the preferred cultivar for exports. The banana industry was saved by the adoption of 'Cavendish' resistant banana cultivars. However, in the 1960s Panama disease emerged in "Cavendish" bananas in Taiwan. This new emerging race is colloquially known as Tropical Race 4 (TR4). TR4 was initially restricted to Southeast Asia region however it has been recently reported in Middle Asia, Africa and re-incurred in Australia. It is likely that it will disseminate further, either through infected plant material, contaminated soil, tools or footwear, or due to flooding and inappropriate sanitation measures. In this study, comparison of re-sequenced and DArTseq data of geographically different TR4 isolates suggested that the temporal and spatial dispersal of TR4 is due to a single clone. This finding underscores the need for global awareness and quarantine campaigns to protect banana production from another pandemic that particularly hits vulnerable small-holder farmers and agricultural based countries.

Mitochondrial genome variation within Fusarium graminearum

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We have sequenced and annotated the complete mitogenomes of 13 geographically diverse strains of the important plant pathogen *F. graminearum sensu stricto* (s.s.). There was no intraspecific variation in the coding sequences of the conserved mitochondrial protein genes, rRNA genes or tRNA genes. We found that presence-absence variation of four group I introns and two homing endonuclease genes (HEGs) accounted for 99.96% of the mitogenome length differences among the strains analyzed. Three group I introns (*cox*2 intron 2, *cob* intron 1 and *cox*1 intron 13) and the HEG encoded by *cob* intron 4 were irregularly distributed among strains of *F. graminearum* s. s. Furthermore, we found that all irregularly distributed introns and HEGs showed evidence of putative horizontal transfer. The patterns of intron/HEG distribution did not correlate to the geographic origin of the strains. Phylogenetic analysis based on the concatenated sequences of of intergenic regions of the mitogenomes showed different tree topology than trees based on specific introns, which is consistent with their different evolutionary histories.

Formae speciales of Fusarium oxysporum can be determined by their putative effector profiles

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Formae speciales (ff. spp.) of the fungus *Fusarium oxysporum* are often polyphyletic in their origin, meaning that strains that infect a particular plant species are not necessarily more closely related to each other than to strains that cause disease in another host. Since isolates of the same *forma specialis* are generally very host specific, at least a section of their genome is expected to be highly similar. De novo whole genome sequencing was performed on wild-type isolates from five different ff. spp. (*cucumerinum, niveum, melonis, radicis-cucumerinum* and *lycopersici)*. For each genome, putative effectors were identified based on small size, secretion signal and vicinity to the 'miniature impala' transposable element. The candidate effector genes of all genomes were collected and the presence/absence patterns in each individual genome were clustered. Members of the same *forma specialis* turned out to group together, with cucurbit-infecting isolates forming a supercluster separate from other ff. spp. Moreover, isolates from different clonal lineages within the same *forma specialis* harboured identical effector gene sequences, supporting earlier evidence for horizontal transfer of genetic material. These data offer new insight into the genetic basis of host specificity in the *F. oxysporum* species complex and show that (putative) effectors can be used to predict host specificity in *F. oxysporum*.

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Nuclear dynamics and chromosome transfer in F. oxysporum

Horizontal transfer of chromosomes contributes to genome plasticity in asexual fungal pathogens. However, the mechanisms behind horizontal chromosome transfer in eukaryotes are not well understood. Here we investigated the role of anastomosis in heterokaryon formation between incompatible strains of *Fusarium oxysporum* and determined the importance of heterokaryons for horizontal chromosome transfer. Using live-cell imaging techniques we demonstrate that conidial pairing of incompatible strains under carbon starvation and nitrogen limitation can result in the formation of viable heterokaryotic cells in *F. oxysporum*. During further development, nuclei of the parental lines presumably fuse at some point as conidia with a single nucleus with both marker histones (GFP- and RFP-tagged) are produced. Upon colony formation, this hybrid offspring is subject to progressive and gradual genome rearrangement. The parental genomes appear to become spatially separated and RFP-tagged histones, deriving from one of the strains, F*o*l4287, are eventually lost. With a PCR-based method we showed that most chromosomes of this strain are indeed lost, leaving hybrid offspring with the genomic background of the other strain (F*o*47) with the addition of transferred chromosome(s) from F*o*l4287, including the chromosome that confers pathogenicity towards tomato.

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Living apart together: crosstalk between the core and supernumerary genomes in Fusarium poae

Eukaryotes can display remarkable genome plasticity, including the presence of supernumerary chromosomes that differ markedly from the core chromosomes. The origin of these supernumerary chromosomes, the reason for their different characteristics, and their interactions with the core genome are still largely unknown. Here we report on the supernumerary chromosomes of the prominent fungal wheat pathogen *Fusarium poae*. Using SMRT long reads, the 38 Mb core genome was assembled into four chromosomes that contain the complete genome complement of related *Fusarium* species in a highly syntenic fashion. An additional ~8 Mb of sequence was assembled into contigs that make up at least one supernumerary chromosome. Clear differences exist between the core and supernumerary genome. The core chromosomes contain 2% transposable elements (TEs) while the supernumerary genome consists of 25% TEs. The TEs on the core chromosomes show clear signs of repeat-induced point mutation (RIP), in sharp contrast no RIP was found on the supernumerary genome. Furthermore, no gene duplications are present on the core, but many are found on the supernumerary genome. Importantly, the specific absence of RIP in the supernumerary genome accounts for the differences between the core and supernumerary genomes in *F. poae*. An exchange of genetic material occurs between the core and supernumerary genomes. Intact TEs from the supernumerary genome integrate into the core chromosomes, occasionally leading to gene disruptions. On the core chromosomes, the integrated TEs are subjected to RIP. In addition, large blocks of supernumerary sequence (>200kb) have recently been translocated to the core chromosomes. *Vice versa*, genes from the core chromosomes are duplicated to the supernumerary genome, where they may show an increase in copy number. This "living apart together" crosstalk bestows significant opportunities for adaptation and evolution on the organism, and shows that the supernumerary genome is an evolutionary cradle for novel genotypes.