Werkgroep Fusarium

Verslag bijeenkomst Werkgroep Fusarium 2023

Like Fokkens

secretaris

Op 8 november 2023 was het weer zo ver: de KNPV werkgroep Fusarium hield haar 36e jaarlijkse bijeenkomst, zoals gewoonlijk in de zaal van het Westerdijk Instituut in Utrecht. De dag was in twee delen opgedeeld. De ochtend werd besteed aan *Fusarium* infecties, met aandacht voor *Fusarium* in eetbare gewassen zoals selderij en sla, maar ook in de sierteelt (Lysianthus), en veevoer (gras), kwantificatie van Fusarium in tannine-rijke wortels, en een reconstructie de evolutie van verschillende levenstijlen (generalist-specialist, pathogeen-endofyt) in *Fusarium*. De middag werd besteed aan moleculair en bioinformatisch genoom onderzoek, met presentaties over *Fusarium*-brede detectie van effectoren, effectoren die gastheer-voorkeur bepalen in *F.oxysporum* isolaten die cucurbits infecteren, de rol van 'accessory chromosomes' in infectie van banaan, en een grootschalige analyse van mitochondriale genomen in *Fusarium oxysporum*. De middagsessie werd afgesloten door de voorzitter van de werkgroep, Anne van Diepeningen, die een overzicht gaf van het onderzoek dat afgelopen jaar gepubliceerd was m.b.t. Fusarium, inclusief nieuwe infecties, maar ook nieuwe resistentie- en (bio)controlemechanismen, en een overzicht van de verschillende congressen over *Fusarium* die we het komende jaar kunnen verwachten. Hierna togen we gezamenlijk naar boven naar de kantine, waar we onder het genot van koffie, thee en heel veel zelfgebakken taart, cake en koekjes, konden na- en bij praten.

De "Fusariumdag" oftewel de jaarlijkse meeting van de KNPV-werkgroep Fusarium (foto: Manon Verweij, Westerdijk Instituut).

Voertaal van de dag was Engels, en een deel van de Engelse samenvattingen zijn hieronder aan het verslag toegevoegd.

De volgende bijeenkomt van de werkgroep zal plaatsvinden op 30 oktober 2024, opnieuw bij het Westerdijk Instituut te Utrecht. Voor meer informatie anne.vandiepeningen@wur.nl of like.fokkens@wur.nl.

Abstracts of the 36th Meeting of the Fusarium working group on next page.

Development of Fusarium wilt resistance in celery and understanding of pathogen virulence factors to underpin biosecurity

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Celery wilt caused by *Fusarium oxysporum* f.sp. *apii* (Foa) causes major losses to celery growers in the USA which is the biggest producer and exporter of the crop worldwide. Spain is Europe's biggest celery producer but so far are no official reports indicating that Foa is present and causing crop loss despite the climate being suitable for the pathogen. There are four known Foa races with races 2 and 4 being the most widespread in the USA. Although plant resistance was developed and successfully used to combat race 2, there is no resistance to the newly emerged race 4 which has also made a host jump to infect coriander.

In a pilot project with Tozer Seeds, we have identified new sources of resistance in their celery diversity set to Foa 4. Moreover, building on Foa genomics work pioneered by Lynn Epstein in the USA, we have also carried out long-read Nanopore genome (re-) sequencing to identify the effector complement in Foa 2 and Foa 4 isolates from the USA as well as *F. oxysporum* f.sp. *coriandrii* isolates from USA and Portugal. This will enable improved diagnostics for Foa 2 and Foa 4 which will be important for identifying potential celery wilt outbreaks in Europe and also add to our understanding of host specificity in *F. oxysporum*.

FoSSy project – Systems approach to control Fusarium oxysporum f.sp. lactucae in leafy vegetables

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Fusarium oxysporum f.sp. *lactucae* (Fol) causes a vascular disease in lettuce that results in significant yield losses. Due to the lack of commercially viable resistant lettuce varieties and restrictions on the use of soil fumigation, there is currently no economically feasible or efficient way to reduce Fol contamination. The FoSSy project was created with the aim of developing a systems approach to strongly reduce and control Fol in the soil-grown lettuce industry. During this project, race-specific and sensitive real-time PCR assays were developed for Fol race 1 and race 4, which are prevalent in Europe. Sample preparation methods were developed for plant tissue, soil, and surfaces, with an extra enrichment step when additional sensitivity was required. The enrichment step focusses on living, and thus potentially infective, fungal propagules, providing a better risk assessment. With the use of these techniques, the epidemiology of Fol in Belgium was evaluated. Results stressed the importance of applying hygienic measures. Inside greenhouses, a patchy distribution of Fol inoculum in the soil could be detected with the presence of Fusarium hotspots. More symptoms were observed in these areas, suggesting a positive correlation between amount of Fol and disease severity. At some lettuce farms, Fol could be detected in the irrigation water, requiring further research. No detection occurred at plant nurseries, implying that these are currently not contributing in the spread of Fol. The real-time PCR assays are also used to determine the efficiency of control strategies to reduce the Fol inoculum in the soil or to evaluate inoculum build-up in tolerant lettuce varieties and alternative crops. Crops grown in rotation with lettuce that support development of the pathogen cause a high risk by maintaining the Fol soil inoculum. Alternative measures are necessary to save the soil-grown lettuce industry. The demand is urgent, as the pathogen itself continues to evolve. This was recently demonstrated by the discovery of the breakdown of (intermediair) resistance to Fol race 4, stressing the importance of an integrated management strategy.

Fusarium oxysporum f.p. lactucae vascular discoloration (Photo: Hanna Mestdagh).

Project partners: Research Station for Vegetable Production (PSKW, Sint-Katelijne-Waver, Belgium), Vegetable Research Centre (PCG, Kruishoutem, Belgium) Inagro (Rumbeke-Beitem, Belgium), Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Merelbeke, Belgium), and Ghent University (Belgium). This project is sponsored by VLAIO and the lettuce industry.

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Exploiting pathogenomics and resistance for the control of Fusarium wilt of lettuce

Fusarium wilt of lettuce which is caused by the soil-borne fungus *Fusarium oxysporum* f.sp *lactucae* (FOL) is responsible for huge economic losses worldwide in both protected and field grown lettuce. Four races of FOL have been identified so far, with race 1 (FOL1) being the most prominent globally and race 2 and 3 confined to Asia. FOL race 4 (FOL4) is newly emerged in Europe and has been reported in Belgium, France, Italy and the UK where up to 70% yield losses can occur. A lack of plant resistance to FOL4 is therefore a major threat to commercial production of lettuce.

The main aim of this project is to characterise new sources of resistance to FOL4 and compare the suite of virulence genes in FOL1 and FOL4 in relation to the response of different lettuce cultivars. So far, resistant lettuce lines have been identified and crossed with susceptible lines to produce mapping populations in a collaboration with Enza Zaden, with the aim of discerning the genetic nature of the resistance. In addition, a potentially important virulence gene (SIX8) with variable sequence was found in FOL4 isolates but was absent in FOL1. Furthermore, an *in vitro* phenotyping system has been developed to carry out RNAseq to examine FOL4 virulence gene expression when inoculated onto susceptible and resistant lettuce cultivars. Additionally, CRISPR Cas9 knockouts of SIX8 in FOL4 showed decreased virulence when screened on susceptible lettuce cultivars. Further work will involve complementation of SIX8 back into FOL4 mutants to further prove its role in virulence.

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Fusarium in Lisianthus cultivation

Lisianthus is one of the important ornamental crops in Dutch greenhouses. Problems with Fusarium in this crop are not new. In the early 2000's the cultivation was affected by *Fusarium avenaceum*. In 2018 *Fusarium oxysporum* fsp. *eustomae* was identified as an important pathogen during a survey of *Fusarium* problems in the greenhouses. Recently, *Fusarium solani* seems to be the main culprit.

Lisianthus is a soil based cultivation, which makes it all the more difficult to get rid of *Fusarium* once it establishes in the soil. It can survive in soil as chlamydospres, which are extremely difficult to eradicate.

Soil steaming between the cultivation cycles is often used by the growers to prevent the spread of the pathogen. It is a very costly management practice. Moreover, it is not always sufficient to prevent the disease. *Fusarium* pathogens are becoming also increasingly resistant to chemical plant protection. Additionally, the availability of the chemical products allowed to be used in the cultivation is also relatively limited. Therefore an alternative management strategies are urgently needed.

Resistant plant material, if available, together with hygiene protocols are the basis of the integrated approach to prevention of *Fusarium* disease. In greenhouse bioassays we have shown that it is also possible to (partially) suppress the pathogen by manipulating the soil microbiome and/or addition of antagonistic microorganism, for example present in biofungicides. Manipulation of natural soil microbiome with addition of specific organic amendments, such as composts, seem to have more robust effect on Fusarium infection. Some biofungicides, used as stand- alone or in combinations, are also effective against this pathogen. However, it remains to be seen to which extend these management strategies can be effective against *Fusarium* in the greenhouse cultivation in practice.

Fusarium species associated with native and forage grasses

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The genus *Fusarium* includes numerous species that have an affinity with grasses, living in an endophytic association, causing diseases, and potentially producing mycotoxins. Native or introduced grasses in Brazil and South Africa can represent inoculum reservoirs of already known or new *Fusarium* species which are potentially plant pathogens, causing loss of yield or quality of crops or livestock. *Fusarium* strains were obtained from forage grasses in Brazil, including *Brachiaria* sp. – frequently used in consortia with important crop plants such as maize and sorghum –, and from about 30 different species of native grasses from the Buffelskloof Nature Reserve in South Africa. The strains were characterized by a multigene phylogenetic analysis using partial nucleotide gene sequences of three gene regions, namely *tef1*, *rpb2* and *CaM*, evaluation of morphological characters, host-substrate preference, and geographic distribution. Nine known *Fusarium* species from three different species complexes were identified from Brazil, and two novel phylogenetic species described within the FFSC as *F. caapi* and *F. brachiariae*. The 50 South African strains belong to 15 distinct *Fusarium* phylogenetic species from eight different species complexes, of which ten represent species so far unknown to science. These results show that native and forage grasses harbor not only a high diversity of known species, which can potentially represent pathogens of crops plants like maize, sorghum, rice and sugar cane, but also novel *Fusarium* species, which deserve our attention for being potential mycotoxin producers and/or plant pathogens.

Key Words: endophyte; molecular phylogeny; new taxa, plant disease; species diversity.

The grass Echinochloa crus-galli and its Fusarium infection (Photo Marileide Moreira Costa).

Quantification of infection in planta

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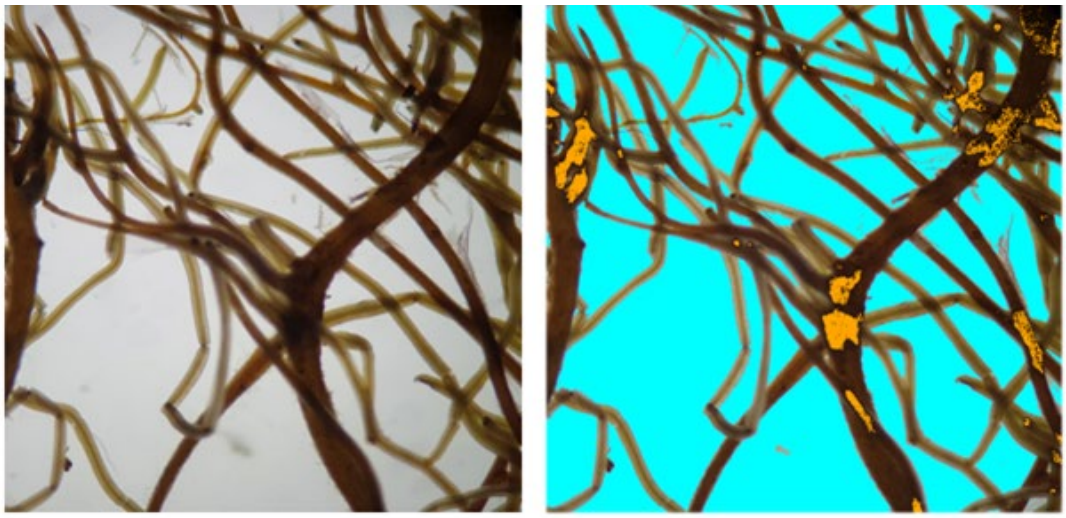
The process of analyzing fungal infection in the roots of raspberry is challenging, partly due to the high lignin content of the roots, but also to analyzing photos using grids absorbs a lot of time. The research done is twofold: optimizing and adjusting the mycorrhiza staining protocol for opportunistic fungi in the lignin-rich raspberry roots next to the automatic analysis of root pictures to identify the % colonization of roots. By varying the exposure temperature and duration of clearing roots, it is possible to make a good contrast between healthy and diseased roots. The quantification process is standardized using an image analysis pipeline, images are gathered and analyzed through an application written in R. Adjustment of the camera and background will always be needed next to negative and positive controls. In the case study, it was not feasible to predict the disease incidence based on root colonization due to the significant differences in colonization within the roots.



Quantification of Fusarium infection in raspberry roots. Image analysis and parameter selection. A) Picture of infected roots; B) Mask applied to the background and to the stains. Root density = 1 – (fraction of background). Root colonization = fraction of stain mask/ root density (Photos Willemijn Steijsiger).

The evolution of lifestyles in Fusarium and allied genera

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The *Nectriaceae* (*Hypocreales, Sordariomycetes*) includes saprobes, endophytes and numerous important pathogens, several of which are of high commercial interest. Members of *Nectriaceae* are circumscribed by having yellow, orange-red to purple, uniloculate ascomata, and phialidic asexual morphs. Presently there are more than 20 genera in *Nectriaceae* that have a fusarioid asexual morph. This group encompass diverse lifestyles, including plant, human, and animal pathogens or are associated with them, saprobes, lichenicolous species, endophytes, and mycophilic taxa. Following the one fungus = one name initiative, *Fusarium* was chosen over its sexual morph, *Gibberella*. *Fusarium* relates to the F3 clade *sensu* Crous *et al*. (2021). The fusarioid genera in *Nectriaceae* do not only differ in their sexual morphs, but also in their asexual morphology and biology, although their ecology has remained rather unclear.

While genome data are available for numerous species, this has been mostly focused on *Fusarium sensu stricto*, as the genus encompasses most of the economically important species in this generic complex. To compliment this, we thus sequenced, assembled and annotated various genomes from type and reference strains spanning all fusarioid genera as well as closely related genera. Phylogenomic ancestral reconstructions of *Fusarium* and closely related genera in the present study showed that plant pathogenicity is ancestral to all fusarium-like and cylindrocarpon-like genera, and revealed multiple and frequent lifestyle transitions, with some exceptions. Although our results depict fusarioid genera as prolific generalists, several genera appear to be more specialised, being primarily plant pathogens, mycophilic, or insect associated, while endophytism evolved several times, and more recently in *Fusarium*.

Identifying effector genes for detection methods in Fusarium oxysporum

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Fusarium oxysporum species complex is an important group of plant pathogens. Although individual isolates have a narrow host range, the group as a whole has a wide host range, and for many crops it is ranked among the most important pathogens. Thisxdiversity and flexibility are the result of genomic diversity and mobility of genes that may spread across phylogenetically disparate groups. The consequence of this phenomenon is that phylogenetic relatedness is a weak predictor of pathogenicity, and the best predictor is the effector profile of a given strain.

Within the Masterplan Fusarium project, we sequenced multiple representatives of different *formae speciales* to identify putative effector genes that could be used as targets for the development of TaqMan assays. The steps were the following: genome assembly, effector prediction based on homology to an earlier effector set, exploring genetic diversity of the isolates (both phylogenetic and effector-focused), identifying effectors with greatest specificity and sensitivity for the target group, mining the genomes for homologous sequences, and designing TaqMan assays based on the collection of target and non-target sequences.

Using large amount of data for this kind of analysis can improve the robustness of the prediction, but significantly complicates calculations. In our analysis, we incorporated sequence variation besides presence absence and invented a new metric for calculating sequence specificity. Data analysis relies on well curated data, which is difficult to obtain and curate. We solved this issue by closely collaborating with experts of phytopathology and data analysis to see if biological observations correspond with observed patterns. Finally, before designing TaqMan assays all sequences from the genome collection were collected, since pseudogenes or other sequences may share local similarities with effector sequences. This approach allowed us to design selective detection methods for groups where we had a sufficient sample set of the pathogen diversity.

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Pan-genome reveals mitochondrial genome diversity and evolution in Fusarium oxysporum

Mitochondria are essential energy-producing organelles in eukaryotic cells. These organelles encode a distinct genome separated from the nuclear genome. The mitochondrial genome is smaller and has a faster evolutionary rate, making it a useful phylogenetic marker. The *Fusarium oxysporum* species complex (FOSC) is a diverse group of fungi with important plant pathogens that cause disease in over a 100 economically important crops. A previous study analyzing the mitochondria of 61 *Fusarium oxysporum* strains revealed that mitochondrial variants are discordant to the nuclear phylogeny, indicating mitochondrial transfer between isolates. Moreover, this study revealed the presence of three major mitochondrial variants. To date 731 genome sequences of FOSC isolates are publicly available, and we hypothesed that including more *Fusarium oxysporum* strains might uncover additional mitochondrial variants. Here, we analyzed mitochondrial genomes from all publicly available *F. oxysporum* isolates to uncover the mitochondrial genome diversity and elucidate evolutionary patterns driving the discordant phylogeny between mitochondrial and nuclear genomes in FOSC. We obtained a complete mitochondrial genome assembly for 486 isolates and constructed a mitochondrial pangenome using a graph-based pan-genomic approach. This mitochondrial pan-genome revealed limited genetic variation, unique intron expansion, and, unanticipatedly, no additional mitochondrial variants beyond the previously known variants. Ongoing analyses of this mitochondrial pan-genome aims to understand the general evolutionary processes that gives rise to the three mitochondrial variants and can help to elucidate the patterns of recombination between FOSC isolates.

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Accessory chromosomes and effectors in Fusarium spp. infecting banana

Members of the *Fusarium oxysporum* species complex, including those infecting banana, contain accessory chromosomes with distinct characteristics from the conserved core chromosomes. These accessory chromosomes have been shown to be important or even essential for pathogenicity and host adaptation in several *formae speciales*. This importance of accessory chromosomes for pathogenicity is likely the result of the accessory encoded effectors. To investigate the role of the accessory chromosomes and their effectors in the interaction with banana, techniques such as RNA-seq and mutant analysis were performed. Expression data shows a strong upregulation of several novel accessory encoded effectors during different infection timepoints. Furthermore, loss of an accessory chromosome leads to a strong reduction in virulence in Tropical Race 4. Further research is currently being conducted to determine the contribution of individual effectors to the virulence on banana.

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A leap into the unknown: understanding host-jumping by Fusarium oxysporum in cucurbits

Fusarium wilt disease, caused by the fungus *Fusarium oxysporum* (Fo), affects over one hundred plant species, resulting in significant crop losses globally. Pathogenic Fo strains are often host specific, only able to infect one or a few related plant species. Fo. f. sp *melonis* (Fom) and Fo f. sp. *cucumerinum* (Foc) are host-specific Fusarium strains that cause disease in melon and in cucumber, respectively. In contrast, Fo f. sp. *radicis-cucumerinum* (Forc) can infect three different hosts within the cucurbits: cucumber, melon and watermelon. In previous research, the first 'non-host' avirulence gene was found in Fom, *Effector Candidate for Cucurbits* (*ECC1a*^{Fom}). Transferring this gene, which encodes a small secreted protein, into a Forc strain compromised its ability to infect cucumber. Based on these findings, we aim to determine plant responses that control compatibility with cucurbit-infecting Fo isolates and to uncover how Fo evolved compatibility towards different cucurbit species. To identify the role of *ECC1a* and its homologs in host compatibility, knock-out mutant strains of Fom and Forc were generated using a CRISPR/Cas9-mediated genome editing approach. The mutants were tested for altered virulence on cucurbits. Preliminary results indicate that *ECC* genes are promising effector candidates to be involved in Fo virulence on different cucurbit species.

Advances in and of Fusarium

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An overview is given of recent research on *Fusarium* worldwide: This includes recent descriptions of human infections ranging from onychomycosis to disseminated infections. Spread of known plant pathogens to new regions like the spreading of *F. oxysporum* f.sp. *cubense* TR4 to the Comoros and further spread in South-America. But, also the spread of some pathogens and concomitant mycotoxins within Europe. Several reports were published on *Fusarium* pathogens breaking their host's resistance. On the other hand also new resistance (mechanisms) are described. To mitigate *Fusarium* problems reports are made on iron deprivation to reduce chlamydospore germination, on volatile organic compounds for the treatment of seeds or to reduce infection. Biocontrol reports have been published on micro-organisms like *Streptomyces* and Pseudomonad species and on the fungus *Piriformospora (Serendipita) indica*. Furthermore, there is some work done on elicitors, on microbiomes and quite some reports on the efficiency of different types of nanoparticles.

Miconazole nitrate is generally not an effective drug of onychomycosis due to Fusarium spp. Vertical streak crème with miconazole, horizontal streak Fusarium proliferatum (Photo Anne van Diepeningen)..