

Dissection of lineage specific chromosomes in the onion pathogen *Fusarium oxysporum* f. sp. *cepae* and other emerging UK pathosystems

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F. oxysporum f.sp. *cepae* (FOC) is a major constraint to onion production worldwide, leading to basal rot of bulbs with estimated losses of £11M per annum in the UK alone. The genetic basis for pathogenicity in FOC was investigated through comparative genomics of multiple pathogenic and non-pathogenic strains. This allowed identification and characterisation of lineage specific regions. Known SIX gene homologs were identified, as well as novel effector candidates, with transcriptome sequencing allowing prioritization of highly expressed effector candidates for functional validation. We observed that core chromosomes within the FOC genome are enriched for genes encoding secreted proteins and cell wall degrading enzymes. Despite this, it is the effectors that are present on lineage specific regions that show greatest expression in planta at 72 hpi, highlighting the role of the LS regions in host

specific pathogenicity. This partitioning of effectors supports the concept of a 3-speed genome in *F. oxysporum* ff. spp..

Expansion of this work into other ff. spp. has led to the identification and characterisation of LS regions in stocks, daffodil, static, pea and lettuce pathogens. This has allowed identification of sets of mimp-associated secreted proteins that show presence/absence variation or sequence variation between *F. oxysporum*. These have proven promising targets to resolve ff.spp. and races within ff.spp. through qPCR, LAMP assays and metabarcoding. This work provides greater understanding of the structure of LS regions within *F. oxysporum* as well as new molecular diagnostics for screening and management of these pathogens at the species-, ff.spp.- and race-level.

Differences in pre-harvest practices linked with the occurrence of *Fusarium* species and associated mycotoxins in maize from two ethnic groups in Vietnam

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Ethnic groups often imply different crop management practices. However, the impact of these different management practices on plant pathogens has never been investigated. Therefore, we investigated the impact of different pre-harvest practices of two ethnic groups of Vietnam on the occurrence of *Fusarium* species in maize. Field maize samples were collected from Kinh farmers and Ede farmers in autumn-winter (AW) crop 2017 (n=6), summer-autumn (SA) crop 2018

(n=9) and AW 2018 (n=16). Beside a questionnaire on agricultural practices, a detailed survey was conducted on occurrence of *Fusarium* species and their mycotoxins in each household. The data indicated that the incidence of *Fusarium verticillioides* contamination in maize grains grown by Ede farmers was higher than in maize grains grown by Kinh farmers. By contrast, the levels of fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), fumonisin B₃ (FB₃), and total fumonisin (FB) in Kinh's maize was less than in Ede's maize. Interestingly, notwithstanding we consider weather as a confounding factor in our analysis, it was remarkable that differences in *F. verticillioides* occurrence and FB₁, FB₂, FB₃, FB contamination correlated with the pre-harvest practices, such as variety, crop rotation, cropping system, tillage practice, crop residue management, and pest management.

Biology, control and detection of *Fusarium* diseases in UK horticulture

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Our research has focussed on four *F. oxysporum* pathogens causing basal rot of onion (f.sp. *cepae*; FOC), lettuce wilt (f.sp. *lactucae*; FOL), Narcissus basal rot (f.sp. *narcissi*, FON) and wilt of column stocks (f.sp. *matthiolae*; FOM). All of these pathogens have caused increasing problems for UK growers over the last decade with the exception of FOL, where a new race of the pathogen (race 4) emerged very recently in 2017. Using genome sequencing and bioinformatics approaches, we have shown that FOC, FOL, FOM and FON genomes are organised into core and lineage

specific regions as in other *F. oxysporum* f.spp. and also identified the range of putative genes involved in virulence and host-specificity. This has led to the development of PCR-based diagnostics to understand pathogen dynamics and potentially assess disease risk. Experiments have also been carried out to define the relationship between *F. oxysporum* inoculum level and subsequent disease development.

Management of *F. oxysporum* pathogens is challenging as they produce long-lived chlamydospores and there are few control options available. We have therefore developed robust plant screening assays and identified new sources of resistance to both FOC and FOL within unique onion and lettuce plant diversity sets at Warwick in order to provide pre-breeding material for industry.



Symptoms of *F. oxysporum* infections in different host plants.

Fusarium poae reduces *Fusarium graminearum* infection and mycotoxin production

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Fusarium graminearum is considered the main causal agent of Fusarium head blight (FHB). However, the disease often comprises regionally specific species complexes. In Western-Europe, *F. graminearum* and *F. poae* are the predominant species present in symptomatic ears. *F. poae* as a weak pathogen is unable to cause FHB symptoms so its omnipresence in symptomatic ears is unexpected.

Here, we demonstrate the intricate relationship between both of them. Controversially, pre-inoculation of *F. poae* reduced the infection ability of *F. graminearum* on wheat leaves and ears. Bioassays showed that inoculation of *F. poae* 1 day and 2 days before inoculation with *F. graminearum*, reduced the number of necrotic spikelets up to 26% and 16%

respectively. Furthermore, using a detached leaf assay, significantly smaller necrotic lesions were found in leaves which were pre-inoculated with *F. poae* and subsequently with *F. graminearum*, compared to a sole *F. graminearum* inoculation. Interestingly, after co-inoculation of *F. poae* and *F. graminearum*, there was no effect on *F. graminearum* biomass but the biomass of *F. poae* increased. Additionally, we found that *F. graminearum* produced less deoxynivalenol (DON), 15-Acetyldeoxynivalenol (15ADON), 3-Acetyldeoxynivalenol (3ADON) and Deoxynivalenol-3-Glucoside (DON3G) when leaves or ears were pre-inoculated with *F. poae*. To investigate this interaction at a plant defense level, expression analysis of salicylic acid (SA) and jasmonic acid (JA) biosynthesis genes on both

leaves and ears was done. These experiments showed that a single *F. graminearum* infection and a co-inoculation of *F. graminearum* and *F. poae* resulted in a consistent down regulation of SA- and JA- responses. On the contrary, although *F. poae* does not induce symptoms, a sequential upregulation of ICS and LOX genes was observed at several timepoints after the inoculation. Using a PCA approach, we demonstrate that pre-inoculation of wheat with *F. poae* one or two days prior

to a *F. graminearum* infection resulted in a typical *F. poae* LOX and ICS expression profile while the suppression of LOX and ICS, which was observed in the single *F. graminearum* infection was not observed. We hypothesize that the early induction of SA- and JA- related defenses by *F. poae* hampers a subsequent *F. graminearum* infection. This study provides new insights in the complex interaction of two predominant pathogenic species in the FHB disease complex.

European Union Reference Laboratory for mycotoxins & plant toxins in food and feed

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Wageningen Food Safety Research hosts the European Union Reference Laboratory mycotoxins & plant toxins in food and feed since March 1, 2018. Tasks and activities of the EURL, as laid down in Regulation (EU) 2017/625, are to provide technical and scientific assistance on analysis to the National Reference Laboratories in the EU Member States and to the European Commission.

Legal limits on mycotoxins and plant toxins in food and feed in the EU are laid down in Regulation (EC) No 1881/2006, Directive 2002/32/EC and Recommendation 2006/576/EC and their amendments. EU regulation on mycotoxins focusses on aflatoxins, citrinin, deoxynivalenol, ergot sclerotia, fumonisins, ochratoxin A, T-2/HT-2

toxin and zearalenone in food and feed and patulin in food. Extended EU legislation is foreseen for the group of *Fusarium* mycotoxins deoxynivalenol, 3- and 15-acetyl-deoxynivalenol and deoxynivalenol-3-glucoside and T-2/HT-2 toxin in food. Furthermore, legal limits or extension of matrices are under discussion for the mycotoxins citrinin in red yeast rice food supplements, ergot alkaloids, ochratoxin A and *Alternaria* toxins.

The work program of the EURL for mycotoxins & plant toxins will be discussed during the meeting. Background will be given on tasks and responsibilities of the EURL, how the work program is designed, focus on compounds and which methods will be developed and extended.

Global epidemic of Panama disease on banana is caused by a new fungal species originating from Southeast Asia

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Spatiotemporal origins and factors impacting dissemination remain elusive for many epidemics. Bananas are the world's most popular fruit and represent crucial food commodities. Global banana production is dominated by Cavendish monocultures, the remedy to manage the Panama disease epidemic in Central America in the last century. Here we will discuss the most recent results of our ongoing efforts to study the diversity and dissemination of the causal agents of Panama disease worldwide. We genotyped a global collection of fungal *Fusarium* isolates and traced

the origins of Panama disease to Southeast Asia, bananas' center of origin and a biodiversity hotspot for banana-infecting *Fusarium* species. While the previous epidemic was caused by a suite of genetically diverse *Fusarium* species, we show that the current epidemic that devastates Cavendish is caused by a single, genetically distinct new *Fusarium* species, and ongoing ingression into banana-growing regions of Asia, the Middle East, the Indian subcontinent, Africa and most recently Latin America seriously threatens worldwide banana production.

Epidemiology of *Fusarium* in greenhouses

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Nearly every crop, either grown in greenhouse or open systems is threatened by one or more *Fusarium* pathogens. Studying the epidemiology of the pathogen is essential for rational control strategies. The mix between different control strategies such as resistance breeding, biological control, hygiene measures, disinfection, crop rotation or fungicides, critically depends on understanding the distribution and the patterns of dissemination, survival, colonialization as well as disease expression.

The three main *Fusarium* pathogens observed in greenhouses on crops are *F. oxysporum*, *F. solani*, and *F. proliferatum*, which are presumed asexual, homothallic and heterothallic, respectively. These species produce different combinations of asexual

micro- and macroconidia, chlamydospores and/or sexual ascospores. Using fluorescently labelled marker strains, we can study the interaction between pathogen and host in more detail. Surveys in three greenhouse crops (chrysanthemum, lisianthus and gerbera) were conducted to study the spread within and between greenhouses. Our results demonstrate that especially soil and plant remains carry high densities of spore inoculum and therefore may play an important role in (re)contaminations. In (recycled) water especially *F. oxysporum* may be observed, while drainage water carries many *Fusarium* species. Aerial distribution seems to play a limited role, but under high infection levels spores of the three examined species may be encountered.

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KNPV heeft in samenwerking met Wageningen Universiteit, NIOO en CSE, een gezamenlijke website gemaakt in het kader van het Jaar van de Plantgezondheid. Informatie en (on line) activiteiten worden op deze site vermeld. Bekijk de site op: www.plantgezondheid2020.nl.