Werkgroep Fusarium

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Fusarium: One fungus, One name (1F1N)

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The genus *Fusarium* includes species of phytopathological, medical, toxicological as well as general interest, attracting researchers with many different backgrounds. Classical nomenclature, where teleomorphs have priority over anamorphs and the fact that many important *Fusarium* species being asexual, have frequently led to confusion across disciplines.
Similar to scientific communities working with important genera like *Aspergillus*, 66 authors representing a large proportion of the *Fusarium* community recently proposed to use *Fusarium* as the basal concept. This will free researchers from the obligation of using other names and eventually lead to reduced misperception among agronomists. Based on the (partial) sequences of the genes coding for both subunits of the RNA polymerase II-B, RPB1 and RPB2, the genus shows well-supported monophyly, with 20 strongly supported species complexes.
Diversification could be mapped on a geological time scale dating back to ~90 Mya. Acquisition of the ability to produce various secondary metabolites maps to more recent dates, the production of trichothecenes being the most recent event around 25 Mya.

First Report of Fusarium oxysporum f. sp. cubense (Foc) Tropical Race 4 associated with Panama disease of banana outside Southeast Asia

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Panama disease of banana, is among the most destructive plant diseases. Race 1 of Foc ravaged 'Gros Michel'-based export trades until the cultivar was replaced by resistant Cavendish cultivars. However, a new variant of Foc, tropical race 4 (TR4), was identified in Southeast Asia and has spread throughout the region. Cavendish clones are the most important in subsistence and export production, and there is a huge concern that TR4 will move into Africa and Latin America. In Jordan, Cavendish bananas are produced on 1,000-1,500 ha. In 2006, symptoms of Panama disease were observed in these plantations and seven isolates were recovered from infected xylem. All examined monospore isolates were placed in VCG 01213, which contains only strains of TR4. Total DNA was extracted from six isolates and PCR analyses were performed, which confirmed their identity as TR4. Subsequently, one of the isolates (JV11) was analyzed for pathogenicity. Root-wounded 10 week-old plants were inoculated by dipping of the Cavendish cv. Grand Naine. Sets of three plants were each treated with either JV11 or two TR4 controls (II-5 from Indonesia and one from The Philipines; both were diagnosed as TR4 by PCR and pathogenicity analyses). Control sets were either treated with race 1 or water. Plants inoculated with JV11 and TR4 controls produced typical symptoms of Panama disease. After 4 weeks, tissue was collected from all plants and plated on Komada's medium. TR4 was directly confirmed by PCR, either directly from symptomatic plants or from isolates that were recovered from these plants. Nothing was reisolated from race 1 inoculated plants and water controls. This is the first report of TR4 affecting Cavendish outside Southeast Asia. It is its northernmost outbreak, and represents a dangerous expansion of this destructive race.

Fusarium infections in a tropical dermatologic clinic

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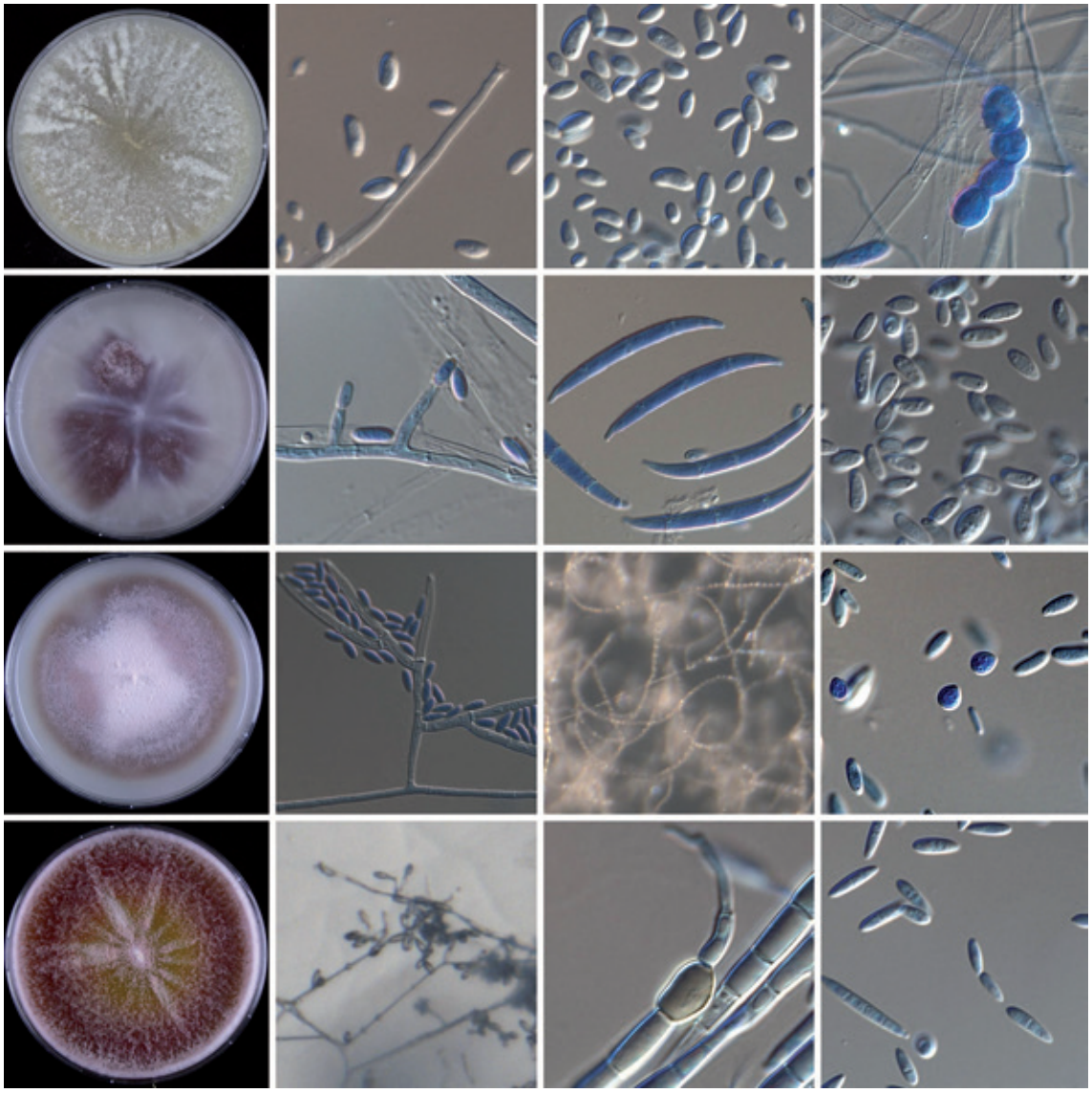
Fusarium species are among the emerging and persistent causative agents of superficial, locally invasive and systemic infections in humans. Superficial infections may be the origin of later deep infections when the host is immunocompromised, then with high mortality. Our objective was to study the prevalence and genetic diversity of *Fusarium* species in a dermatological ward in Thailand. Approximately ten percent of the infections proved to be due to *Fusarium* spp. based on multilocus DNA sequence-based genotyping using partial sequences of elongation factor 1-apha (EF1), RNA dependent polymerase subunit II (RPB2), and the internal transcribed spacer (ITS) region. The majority of the 44 *Fusarium* isolates belonged to the *Fusarium solani* species complex (FSSC), one strain matched with the *Fusarium oxysporum* haplotype 33 known from multiple human infections, while six others belonged to the *Fusarium incarnatum-equiseti* species complex (FIESC). No members of the *Fusarium fujikuroi* species complex (GFSC) or *Fusarium dimerum* species complex (FDSC) were detected, though they have been the etiological agents of similar infections around the world. Within the FSSC two predominant sequence types could be recognized; one particular cluster proving to be BSL-2 *Fusarium falciforme* (previously known as *Acremonium falciforme*, bsl-2), the other the recently described *Fusarium keratoplasticum*. Nail and skin infections were prevalent. Infections probably started by environmental strains after damage to nails or small wounds.



Members of the Fusarium solani species complex (top row), Fusarium oxysporum species complex (second row), Fusarium fujikuroi species complex (third row) and Fusarium chlamydosporum (bottom row): each of these species that may cause infections in humans.

Human infections by members of the Fusarium fujikuroi species complex

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We describe the first human case of a fatal disseminated infection in a leukemic patient, caused by a member of the *Fusarium fujikuroi* species complex (FFSC), viz. *Fusarium andiyazi*. This species was thus far only known as plant pathogen. In general, human infections by *Fusarium* species range from infections of nail, skin and eyes in healthy individuals, to disseminated infections in immunocompromised patients. The disseminated infections have a relatively high mortality and according to a European study are caused mainly by members of the *Fusarium solani*, *F. oxysporum*, and *F. fujikuroi* species complexes. This last group is treated here in more detail.
Developments in molecular phylogenetics based on multi-gene analyses have shown that the *F. fujikuroi* clade includes at least 50 species. However, many of these species are difficult to distinguish from each other morphologically. Most species occur often world-wide and have broad ranges of host plants. To date at least 11 FFSC species have been reported to be able to cause human infections. Many of the FFSC etiological agents prove to have high intrinsic resistance to available antifungal drugs. Faster accurate diagnostic tools are therefore needed.

Antifungal susceptibilities within the Fusarium genus and use of MALDI-TOF MS

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Objectives: (1) Evaluate the *in vitro* antifungal susceptibility profiles of clinically important *Fusarium* species, by performing EUCAST E.DEF9.1 broth microdilution, with commonly applied antifungal drugs, on the, recently re-identified and validated, *Fusarium* strains of the Belgian fungal BCCM/IHEM collection. (2) Analyze the epidemiological data available for each of these strains. (3) Assess the capacity of MALDI-TOF MS to correctly identify these *Fusarium* strains.
Results and conclusions: Due to variability in susceptibility profiles between species, correct identification at species-level appears advisable for *Fusarium* infections to improve antifungal therapy, and MALDI-TOF MS has the capacity to do so (94,4% correct identifications). Our study also showed an interesting similarity between the *Fusarium* distance matrix of MS-spectra and the ITS-BT-EF1 -LSU Bayesian phylogeny. Additionally, we observed that several important species were highly sensitive for terbinafine (more than voriconazole) and that posaconazole had no effect against any *Fusarium* species. Amphotericin B remains the most suitable drug when identity of the infecting *Fusarium* species is unknown, nevertheless susceptibility has decreased over the last 30 years for *F. oxysporum* and *F. verticillioides*. Alarming is that these latter species seem to cause more often disseminated and locally invasive infections than *F. solani*, until now considered the most virulent species.

A multi-disciplinary approach to study the Fusarium diversity in Chinese maize and wild banana.

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The genus *Fusarium* includes numerous species that are spread worldwide. Until today informative *Fusarium* surveys from Europe and US are available, while less is known about China, except for *F. oxysporum* and *F. graminearum* infecting cultivated banana and cereals respectively.
For this reason four field collections were performed in four different Chinese Provinces (Hainan, Henan, Hubei and Yunnan), from 2009 to 2013, with the aim of studying *Fusarium* diversity on maize and wild banana.
The isolates were identified combining morphological and molecular analysis, the latter using PCR amplification and sequencing of EF-1a and ITS genes for *Fusarium*.
The attention was directed to species from the *Fusarium fujikuroi* Species Complex (FFSC) present on wild banana and from the *Fusarium incarnatum- equiseti* Species Complex (FIESC) present on maize and wild banana. In order to have a deeper understanding of these species we decided to use a multi-disciplinary approach. Phylogenetic analysis, study of the mycotoxin production profile and the presence of the fumonisin/tricothecenes gene clusters have been investigated and some experiments are still ongoing.
Furthermore FFSC and FIESC isolates are currently tested for their aggressiveness on banana fruits or maize plantlets and also for their resistance to fungicides.

Genetic mapping of resistance to Fusarium oxysporum in tulip

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Fusarium oxysporum is a major problem in the production of tulip bulbs. Agronomic measures such as avoiding wounding, removing diseased bulbs and crop rotation are insufficient to control the disease and therefore the control strategies rely on the frequent use of chemicals. The chemical use causes environmental risks and the prolonged use is likely to lead to fungicide resistance in the pathogen. Breeding for *Fusarium* resistant tulip cultivars is therefore an attractive alternative and a main target for breeding companies. Unfortunately, tulip has a long life cycle (4-5 years) and therefore conventional breeding is a slow process. Marker-assisted breeding has the potential to speed up the breeding process considerably. Obstacles for this approach are the large genome size (1C≈30GB) for which few molecular marker have been published, and the absence of a genetic map. To remove these obstacles, we constructed the first genetic linkage map for tulip and searched for markers linked to resistance to *Fusarium*. A mapping population segregating for *Fusarium* resistance was genotyped using SNP, AFLP, NBS and SSR markers which were used to generate two parental maps. The mapping population was phenotyped in two consecutive years by soil infection tests, in which the infection degree was scored visually on a 1-5 scale. In addition, we used a GFP (Green Fluorescent Protein) tagged *Fusarium* strain to monitor and quantify the infection on the mapping population using the newly developed PathoScreen platform. We demonstrate that *Fusarium oxysporum* resistance in tulip is a quantitative trait for which five putative QTLs were identified derived from both the resistant and susceptible parent.

The use of green leaf volatiles as additional tool in integrated pest management against Fusarium Head Blight

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Biogenic Volatile Organic Compounds (BVOCs) are known regulators of the communication of sedentary plants with their direct environment. Next to attracting pollinators, repelling insect herbivores and even possessing antifungal or antimicrobial properties, BVOCs can act as an alarm signal to warn neighboring plants of an imminent herbivorous or pathogen attack. For example, green leaf volatiles (GLVs) can be perceived by other plants, after which defense pathways in those plants are initiated. One type of induced resistance is called priming. A plant in primed state will be able to respond faster and/or stronger to an attack with a pathogen or herbivorous insect than a plant in unprimed state.
In this project, we work with wheat and the predominant species causing Fusarium head blight FHB (*Fusarium graminearum)*. First we identify the BVOCs that are released by wheat plants upon infection with *F. graminearum*. Subsequently, wheat plants are exposed to these BVOCs prior to infection with *F. graminearum*. Samples are collected at different moments post-infection and then analyzed by qPCR for expression levels of a selection of resistance genes.
The data are discussed in relation to the potency of BVOCs to prime wheat by enhancing defense pathways in the protection against *F. graminearum*.

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Evolution of races within f.sp lycopersici of Fusarium oxysporum

Three physiological races (1, 2 and 3) of *Fusarium oxysporum* f.sp *lycopersici* (Fol) have been identified based on their inability to infect tomato cultivars carrying Fol resistance genes (*I*, *I-2* or *I-3*, respectively). We wished to unravel the molecular mechanisms underlying the evolution of Fol races. It is generally assumed that race 2 evolved from race 1 by loss of *AVR1* and that race 3 evolved from race 2 by a point mutation in *AVR2*, thus overcoming *I* and *I-2* mediated resistance, respectively. We have sequenced a genomic region of approximately 100 kb containing *AVR1* in race 1 isolate Fol004 and compared it to the sequenced genome of race 2 isolate Fol4287. A genomic fragment of 31 kb containing *AVR1* was found to be missing in Fol4287. Further analysis suggests that race 2 evolved from race 1 by deletion of this 31 kb fragment, most likely due to recombination between helitrons bordering the fragment. A worldwide collection of 63 Fol isolates was subjected to PCR analysis of the *AVR1* genomic region, including the two bordering helitrons. The results suggest that, based on the deletion event that led to loss of *AVR1*, Fol isolates can be divided into distinct lineages that coincide with their geographical origin. Our results also suggest that transposable elements played a major role in the evolution of races within f.sp *lycopersici* of *Fusarium oxysporum*.

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Identification and functional analysis of virulence genes in Fusarium oxysporum f. sp. cucumerinum

Fusarium oxysporum (*Fo*) is a soil-borne, pathogenic fungus that infects plants through the roots, grows into the xylem vessels of the plant and thereby causes Fusarium wilt disease or root and stem rot. In earlier research in our lab, a number of effector or SIX (Secreted In Xylem) genes were identified. These were later shown to be encoded on Fol's mobile, TE-rich 'pathogenicity-chromosome' that can be transferred horizontally to non-pathogenic *Fo* strains, resulting in acquired pathogenicity. The genomic characteristics and context of these genes are interesting, as these can be used to predict novel virulence genes. In this way, 10 putative effectors were identified in *Fo* f.sp. *melonis* (Fom), most of which are also expressed during melon infection.

We hypothesized that the putative effectors that are used during melon infection may also be used by other, related, cucurbit-infecting *formae speciales*. Using a PCR-based approach, we determined which effectors are present in our collection of *Fo* f.sp. *cucumerinum* and *radicis-cucumerinum* (Foc/Forc) isolates and assessed the virulence of these strains upon infection of susceptible and resistant cucumber cultivars.

We found that one group of isolates in our collection is completely avirulent on resistant host plants. These isolates share the same VCG and RAPD group and are also unique in that all contain Fom putative effector 8. All other Foc isolates (lacking effector 8) were able to cause disease on the 'resistant' cucumber line.

Based on these results, the Fom putative effector 8 gene is a prime candidate for functional analysis. Additionally, we have selected several strains of both Foc and Forc for genome sequencing and RNA-seq to discover additional virulence related genes. Phylogenetic analysis of effectors across *formae speciales* will help to reconstruct the evolution of host-specific pathogenicity in Fo and the dynamics of the mobile accessory genome. Newly discovered virulence genes can be used as molecular markers for diagnostic purposes.